SOME ASPECTS
OF
THE BIOLOGY OF THE TAPEWORMS
PROTEOCEPHALUS SPP. AND *SCHISTOCEPHALUS SOLIDUS* (MÜLLER)

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PROTEOCEPHALUS SPP. AND

SCHISTOCEPHALUS SOLIDUS (MÜLLER)

D. K. GEMMELL

SUMMARY

The thesis is divided into 7 sections, each with a format suitable for individual publication.

In Section 1 the survival of eggs of the tapeworm Proteocephalus filicollis from three-spined sticklebacks Gasterosteus aculeatus is investigated. Eggs remained infective longest at low temperatures. Procercoid development of P. filicollis in the copepod Eucyclops serrulatus is described, and sticklebacks were successfully infected with 39 day old laboratory reared procercoids. While maintenance of the P. filicollis infection in the fish host in the laboratory proved difficult, some success in transferring worms from one fish to another was achieved.

P. filicollis is shown in Sections 2 and 3, by the examination of monthly samples of sticklebacks over a two year period, to lack a seasonal incidence and maturation cycle in two Glasgow sites. A seasonal spatial distribution pattern of plerocercoids within the fish intestine occurred in one of the sites. Strobilate worms in both sites were generally restricted to the anterior gut region. It is concluded that, while the onset of strobilation is not entirely dependent on plerocercoid length, plerocercoids in both sites were most likely to strobilate when the same length.

In Section 4 it is argued that the proteocephalid in powan Coregonus

lavaretus in Loch Lomond is probably a new species. The development of procercoids of this worm in the copepods Mesocyclops leuckarti and Diaptomus gracilis is described.

The proteocephalid infecting powan C. lavaretus is shown in Section 5, by the examination of monthly fish samples over a two year period, to possess a distinct incidence and maturation cycle, adult worms occurring only in summer. There is evidence for the presence of a population of procercoids persisting in copepods throughout the winter.

The host/parasite relationship of larval cestodes and their copepod hosts is studied in Section 6. Procercoids of the proteocephalid from powan C. lavaretus failed to grow and subsequently died in the haemocoels of E. serrulatus speratus and Cyclops albidus. Procercoids of P. filicollis died in the haemocoels of E. serrulatus speratus and Cyclops albidus, death in this case being accompanied by, or assisted by, the formation of a sheath, composed probably of host blood cells, around the dying procercoid. The factors determining host specificity in copepod/procercoid systems is discussed in some detail.

A technique for the production of large numbers of eggs of the pseudophylidean tapeworm Schistocephalus solidus is described in Section 7. Eggs of the worm developed normally in 25% sea water, but abnormally in more hypertonic sea water solutions. The rate of beat of the flame cells of unhatched coracidia was reduced in 25% sea water, and increased after hatching. The copepod E. serrulatus speratus is shown to be yet another suitable host for procercoid development of S. solidus.

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SECTION 1

Studies on the biology of *Proteocephalus filicollis* (Rud, 1810) a cestode parasite of the three-spined stickleback *Gasterosteus aculeatus* L.

(1) Laboratory investigations into the life-history.

(with 8 figures in the text)

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INTRODUCTION

The ecology of the life cycle of Proteocephalus filicollis in Gasterosteus aculeatus has been investigated by Hopkins (1959), Willemse (1968), Chappell (1969) and in Sections 2 and 3. Since a number of important questions remain unanswered the present author decided to attempt to answer them through laboratory investigation.

The only previous investigation of the life cycle of P. filicollis in the laboratory is that of Meggitt (1914) which, in addition to the observations of Hopkins (1959) established that a copepod serves as the intermediate host for procercoid development; there is no second intermediate host, the stickleback acquires the parasite by eating infected copepods.

MATERIALS AND METHODS

1. The egg of Proteocephalus filicollis

(a) Source

Gravid Proteocephalus filicollis were removed from the intestine of Gasterosteus aculeatus caught in a Glasgow pond and canal (see Sections 2 & 3) and transferred to watch glasses containing fresh tap water. Some observations on the behaviour of the oncosphere within the egg after release from the worm were made.

(b) Egg maintenance

1. Short term

Eggs not used immediately were kept at 4°C in covered jars for a few days.

2. Long term

For a study of egg survival at various temperatures, eggs fresh from 9 gravid worms were pooled in 30 ml of tap water and maintained at 12°C for 2 h. The jar was shaken and 2 ml of egg suspension was removed and added to each of 10 2" by 1" glass tubes and topped up to 20 ml with tap water. Perforated aluminium foil caps were placed over each tube. The tubes were placed in the dark in pairs at 25°C,20°C,15°C,10°C and 4°C. Every 2nd or

3rd day 15 to 17 ml of supernatent was removed from each tube and replaced with fresh water already at the correct temperature. A small number of eggs (usually 15 to 20) was removed periodically with a pasteur pipette from one of the two tubes kept at each temperature for infection of copepods.

2. The copepod

(a) Collection of copepods from the field

Eucyclops serrulatus s.s. were collected from the River Kelvin near Glasgow using a zooplankton net. Copepods were separated from debris and other animals by decanting and sieving.

(b) Copepod culture

Eucyclops serrulatus s.s. were cultured in the laboratory using the technique of Orr & Hopkins (1969) slightly modified in that aeration of the tanks was continuous rather than once weekly for 8 h. Seven 14 l tanks of breeding copepods were at one time in use.

(c) Measurement of copepods

Using a thin film of water, copepods with egg sacs were held firmly, ventral surface down, on a glass slide and the distance between the anterior end of the cephalo-

thorax and the posterior of the anal segment (Fig. 1) was measured.

3. Infection of copepods

Copepods were placed in 1 cm of water in crystalising dishes of various diameters to which an excess of eggs of Proteocephalus filicollis was then added. Observations on the behaviour of copepods with respect to the eggs were made. Watch glasses containing approximately 10 copepods were used in the egg survival experiment. Eggs were generally removed after 2 - 4 h by passing the contents of the dishes through a 210 mμ sieve which retained the copepods but not the eggs. The copepods were then returned to water for maintenance.

4. Maintenance of infected copepods

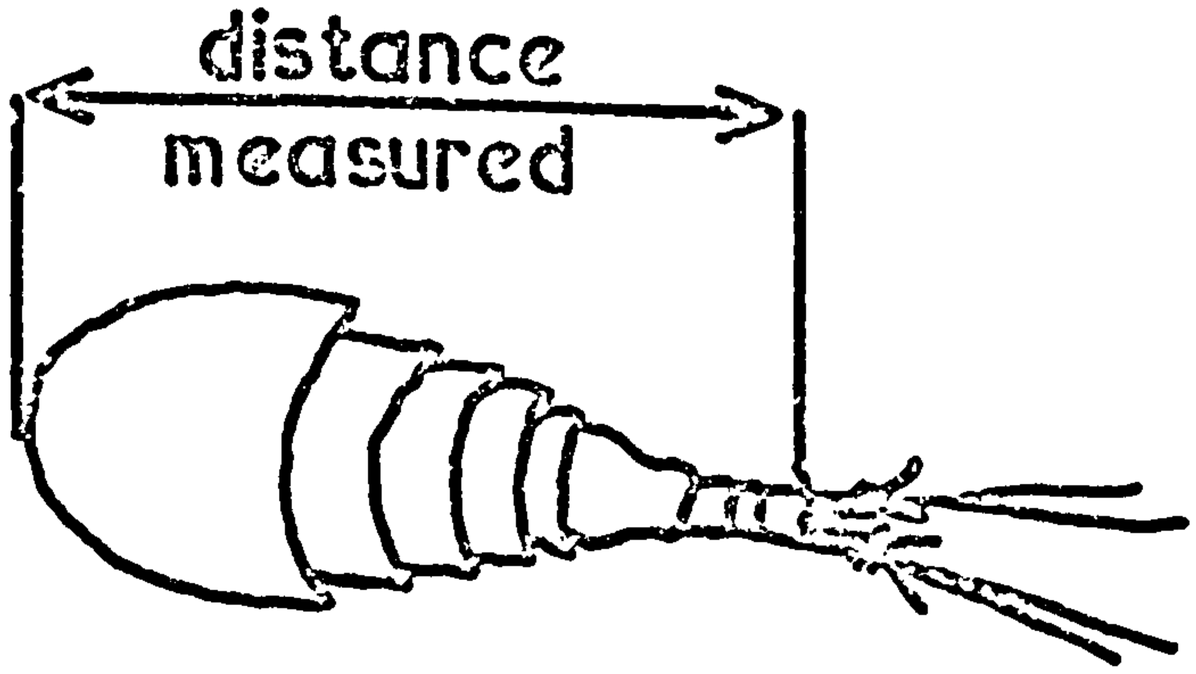
(a) Short term

Infected copepods were kept in water in crystalising dishes of various diameters at temperatures as in the results. Hay infusion, rich in ciliates, was added periodically to each dish. The amount and frequency of infusion addition was dependant on the volume of the copepod containers and the number of copepods. The water in

Figure 1

Female copepod to show the distance measured between the anterior tip of the cephalothorax and the posterior end of the anal segment.

N.B. appendages and egg sacs are not included.



each was completely changed if necessary. Dishes were aereated continuously using a difuser stone.

(b) Long term

To obtain procercoids infective to fish a technique for long term maintenance of infected copepods in river water at 20°C was devised (Fig. 2). Due to the muslin ring escape of copepods from the jar was impossible. After 39 days each copepod was placed on a slide in a thin film of water and the number of fully developed procercoids determined using transmitted light and X50 magnification.

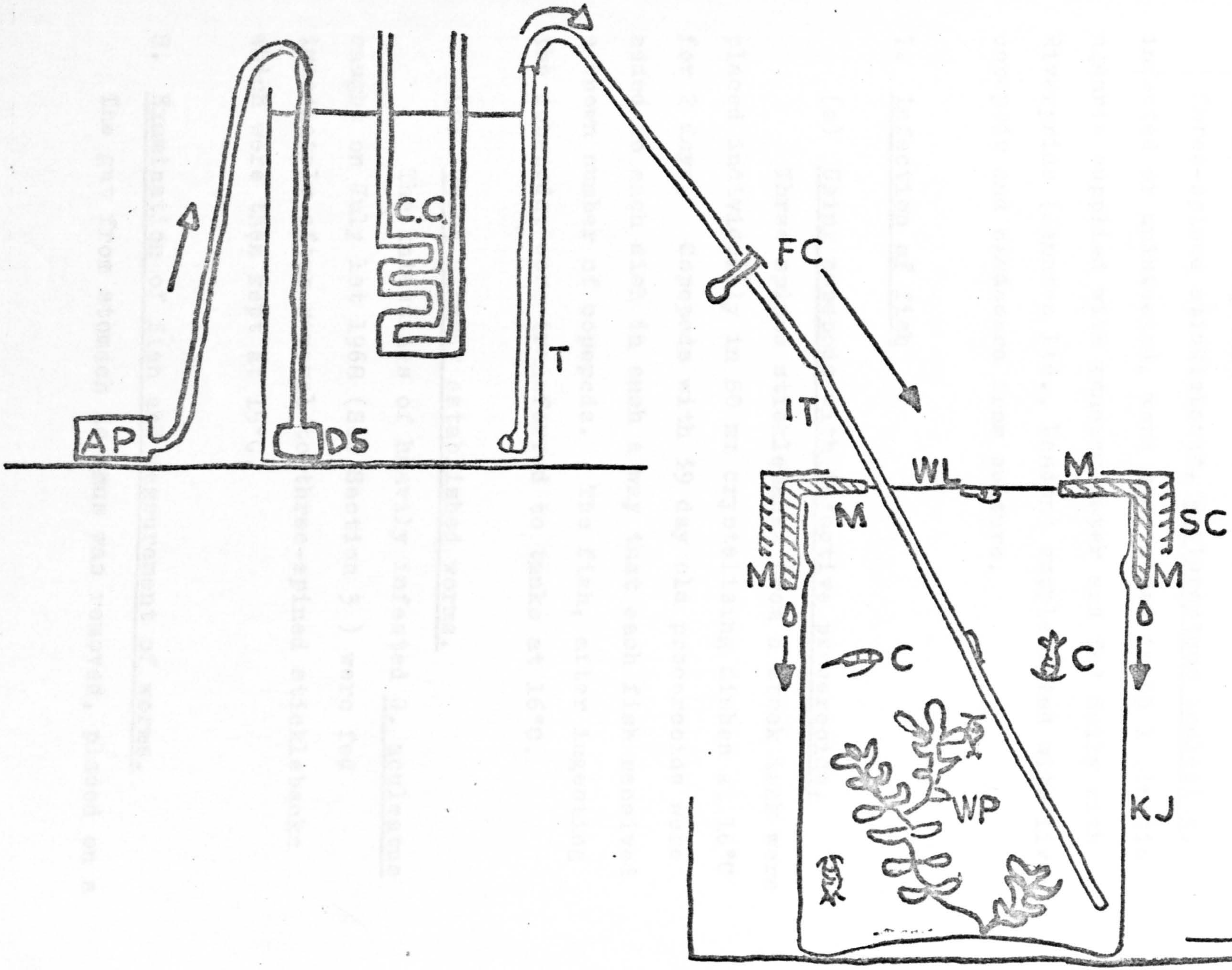
5. Copepod examination and procercoid measurement

Copepods were mounted in water for microscopic examination. By moving the coverslip the copepods could be rolled into any desired position so that the number of procercoids present could be determined. The dimensions of procercoids were measured *in situ* with a camera lucida or micrometer eye piece. Nauplii, being very fragile, were held in a thin film of water on a slide for examination. In the egg survival experiment all copepods were examined within 3 h of exposure to the eggs.

Figure 2

Apparatus used for long term maintenance of infected copepods at 20°C. A.P. = air pump; C = copepod; CC = cooling coil (using tap water); D.S. = diffuser stone; F.C. = flow controller (siphon); I.T. = intake tube; K.J. = Kilner Jar; M = muslin; S.C. = screw cap, T = reservoir tank (river water); W.L. = water level in Kilner jar; W.P. = water plant.

NOT TO SCALE


C.C.
T
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KJ

6. Maintenance of fish

Three-spined sticklebacks, Gasterosteus aculeatus, infected or uninfected, were maintained in 55 l plastic aquaria supplied with running water and fed daily with Riverpride (Jannsen Ltd., London) supplemented with live copepods and cladocera from culture.

7. Infection of fish

(a) Using copepods with infective procercoids.

Three spined sticklebacks from a stock tank were placed individually in 80 mm crystalising dishes at 16°C for 2 days. Copepods with 39 day old procercoids were added to each dish in such a way that each fish received a known number of copepods. The fish, after ingesting the copepods, were transferred to tanks at 16°C.

(b) Using already established worms.

The intestines of heavily infested G. aculeatus caught on July 1st 1968 (See Section 3) were fed immediately after removal to three-spined sticklebacks which were then kept at 15°C.

8. Examination of fish and measurement of worms.

The gut from stomach to anus was removed, placed on a

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8. Examination of fish and measurement of worms.

The gut from stomach to anus was removed, placed on a

3" by 2" slide and examined under slight pressure from a covering 2" by 1" slide using transmitted light and X25 magnification. After noting the position of attachment of any worms present the worms were transferred to water, allowed to relax until dead and measured. Control fish, unexposed to infected copepods or infected guts, yet from the same stock tanks as the experimental fish, were also examined for the presence of <u>P. filicollis</u>.

RESULTS

1. Egg longevity

As shown in Fig. 3 eggs of Proteocephalus filicollis maintained at 25°C for 3 and 5 days failed to infect copepods, dead oncospheres being found in the copepod intestine. Dead oncospheres of eggs kept at 20°C, 15°C and 10°C were first noted in the copepod intestine on days 12, 14 and 16. By 16 days eggs kept at 20°C were uninfective. The eggs at 15°C and 10°C continued to infect copepods until the 26th and 23rd days respectively.

Dead oncospheres of eggs kept at 4°C did not occur in the copepod intestine until the 26th day. Eggs at 4°C retained their infectivity until the 44th day.

2. The development of P. filicollis procercoids in Eucyclops serrulatus maintained at 20°C.

Fig. 4 shows a fully embryonated P. filicollis egg infective to a copepod. Escape of the oncosphere from the surrounding embryophore into the lumen of the outer membrane was only noted under coverslip pressure. Although fairly buoyant, eggs tended to adhere to the base of containing vessels.

From hours of observation it was clear that when an egg of P. filicollis came into contact with the mouthparts

Fig 3 The infection of copepods with eggs of P.filicollis maintained at various temperatures

The black and the clear regions represent periods when copepods after 3 hours had live oncospheres in their haemocoels or dead oncospheres in their intestines. The crosshatched regions represent the period when both oncospheres were present in the haemocoel and dead ones were found in the gut.

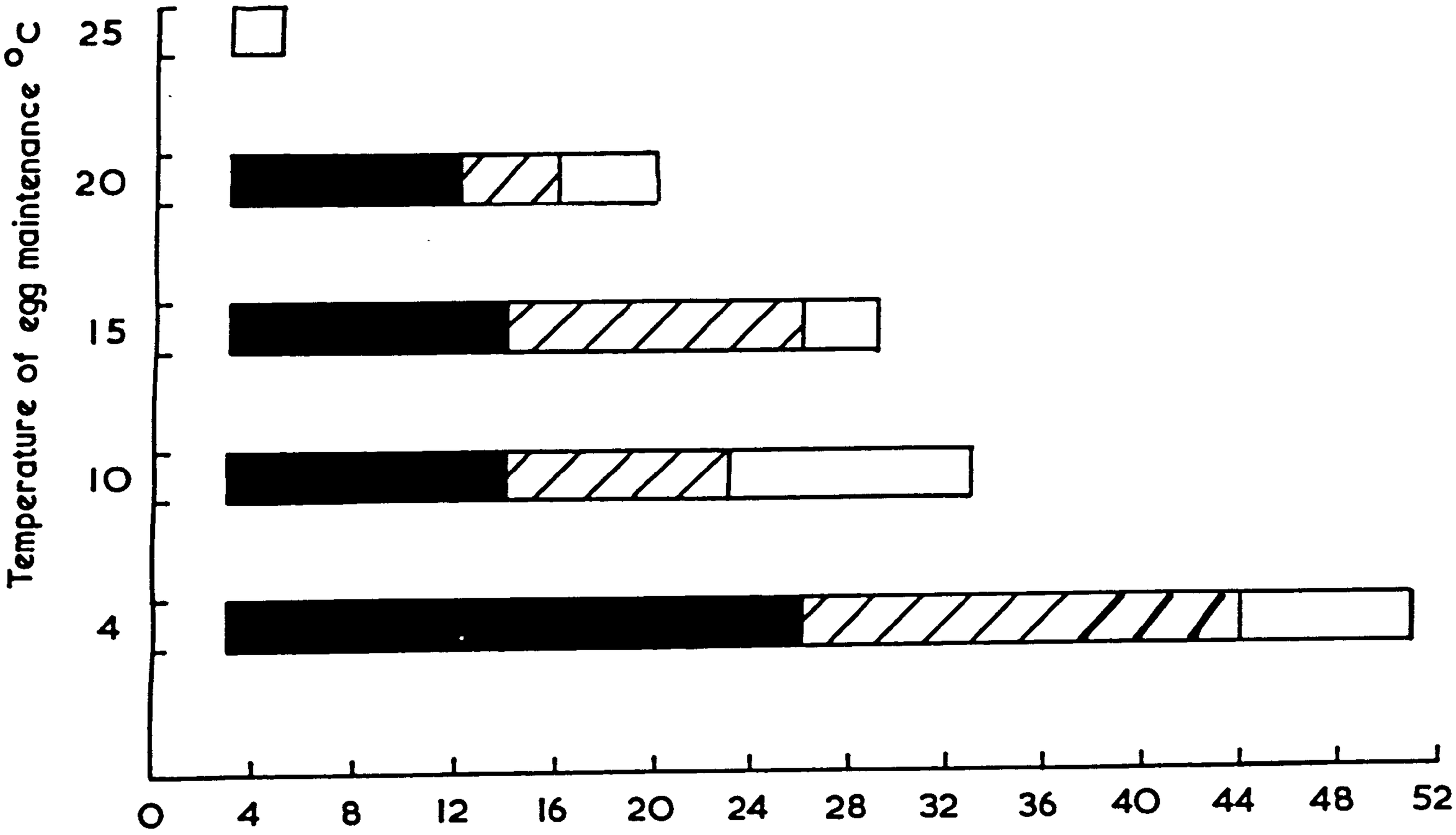
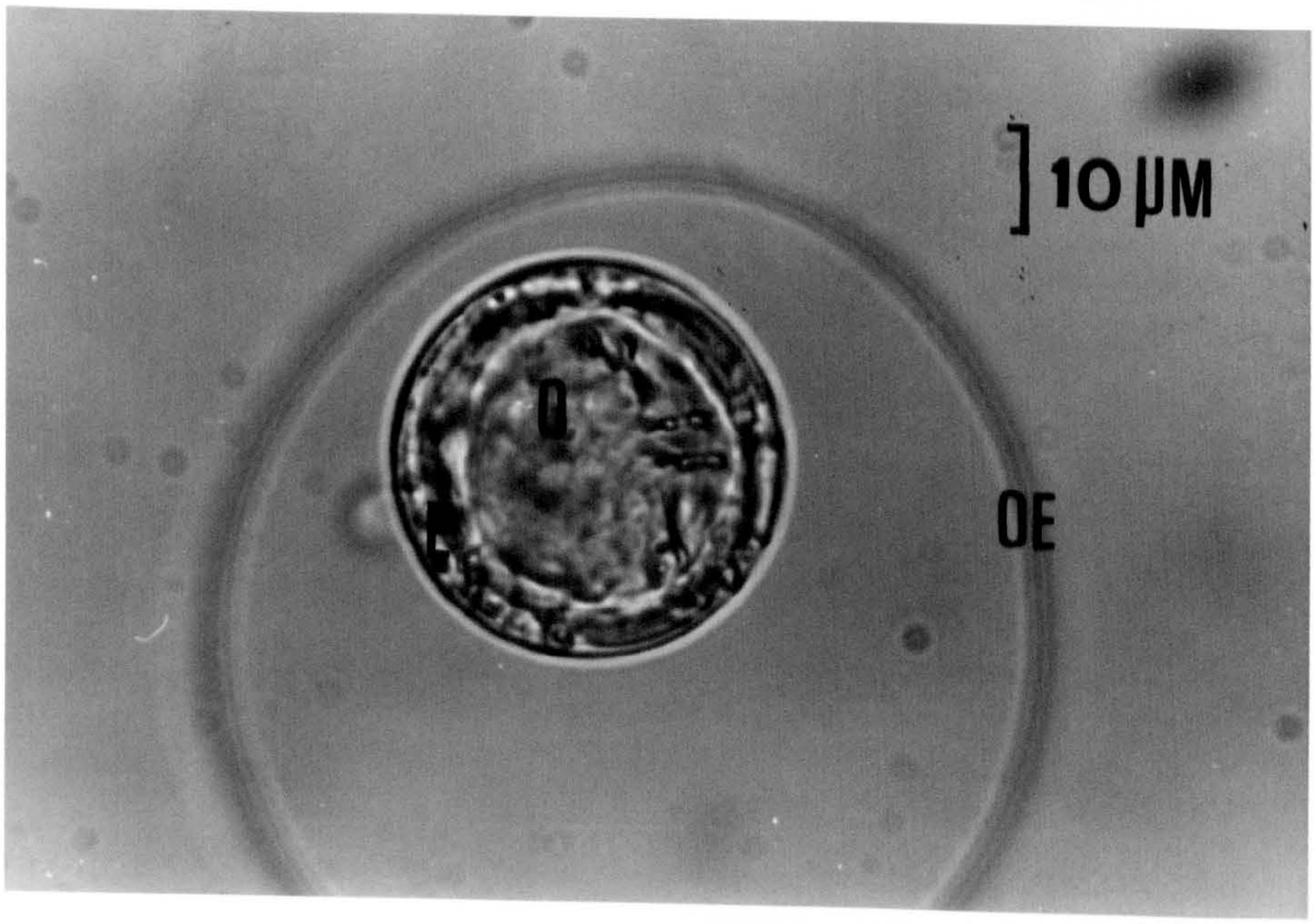


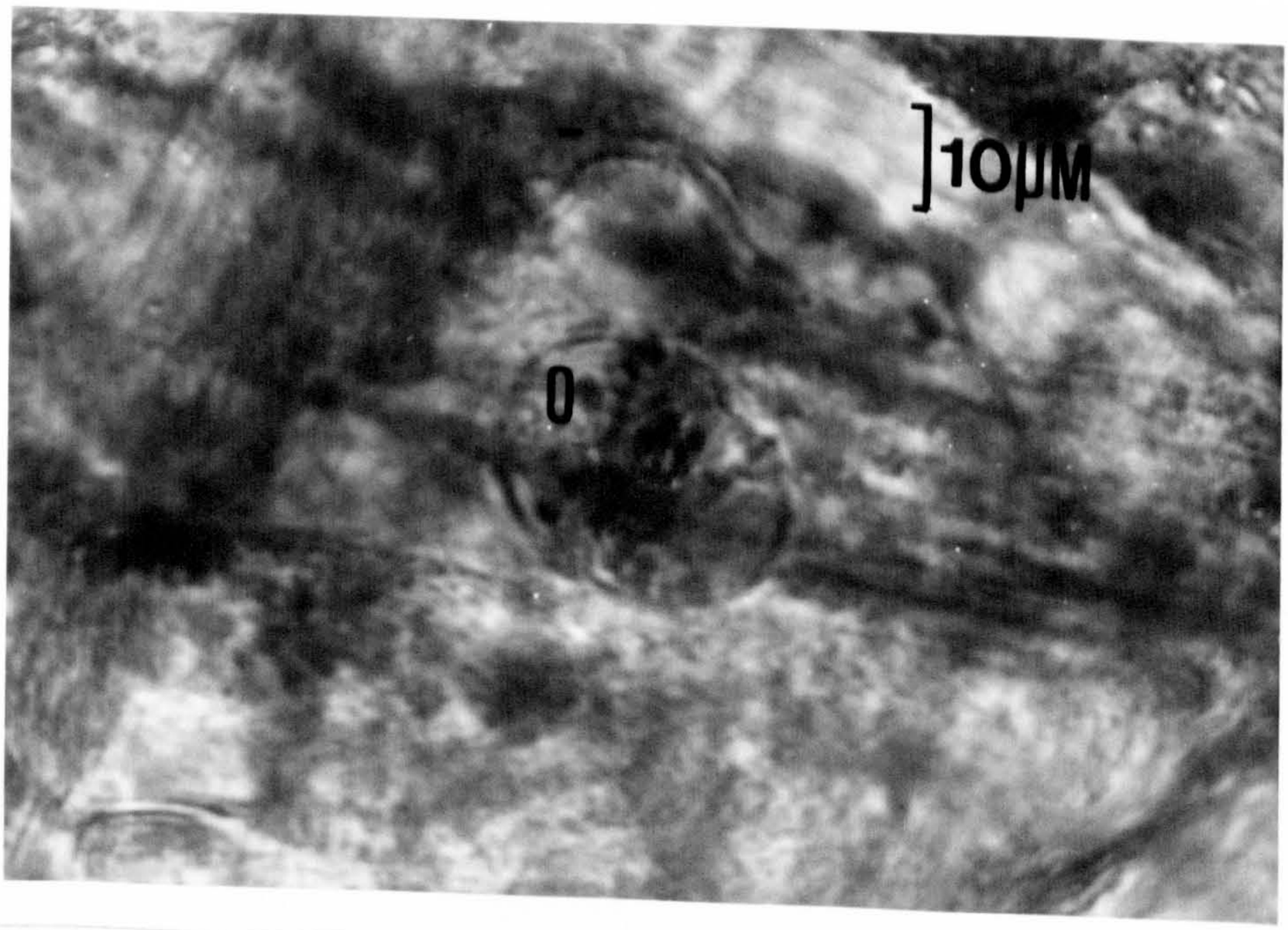
Figure 4

Infective egg of Proteocephalus filicollis showing the oncosphere (O) surrounded by the embryophore (E) and the outer envelope (OE).

Figure 5

Oncosphere (O) of Proteocephalus filicollis in the haemocoel of Eucyclops serrulatus.


10 µM
OE


10µM

of a browsing E. serrulatus it was invariably ingested. The eggs were always swallowed whole. If a copepod, as it browsed, passed close to an egg but made no physical contact with it, that egg was not eaten.

A newly penetrated oncosphere was once noted in the haemocoel of a copepod only 20 minutes after initial exposure to the eggs. In many cases penetration within half an hour to an hour was noted, while the results of the egg longevity experiments just described indicate that penetration was completed within 3 hours. Generally penetration occurred in the mid gut, but was once noted in the hind gut. The intensity and incidence of infection in copepods in the laboratory varied considerably, depending on the relative density of copepods and eggs. The maximum number of young procercoids noted in a laboratory infected copepod was 13, while intensities of from 1 to 5 procercoids per copepod were most common. The small procercoids, for the first few days post-infection were moved rythmically back and forth in the copepod haemocoel by the continuous pulsation of the intestine.

Newly penetrated oncospheres (Fig. 5), approximately 28 μm in diameter, were highly active both the body and the hooks moving rythmically continuously. Between the 3rd and the 12th day the procercoids elongated becoming

sub-spherical and highly vacuolated in appearance, the hooks becoming very difficult to discern. One larva on the 11th day was 121 µm long by 83 µm broad. The constriction off of a cercomer occurred any time between the 15th and 25th days depending on the size and degree of development of the procercoid. Cercomer formation never occurred on larvae less than 200 µm long. Hooks were never found on a cercomer, being left scattered loosely in the main body of the developing procercoid. Calcareous corpuscles first appeared during or immediately after cercomer formation and loss. Suckers were first observed on a procercoid, 264 µm long by 110 µm broad, which had been developing alone in a copepod for 20 days. Suckers generally appeared between the 20th and 30th days. On the 25th day a procercoid measuring 484 µm long by 63.6 µm broad had fully developed suckers. By the 39th day procercoids were used to successfully infect sticklebacks in the laboratory. The newly established plerocercoids, derived from the 39 day old procercoids ranged in length from 280 µm to 600 µm. Once a well developed procercoid was freed from the copepod into water it's scolex frequently invaginated. Scolex invagination was never observed at any stage while the procercoid was still within the

copepod. Fig. 6 shows two procercoids, both 25 days old and derived from the same copepod, one with fully developed suckers and probably infective to a stickleback, the other still with its cercomer attached.

3. Infection of, and procercoid development in, nauplii, copepodites and adults of E. serrulatus.

Nauplii, copepodites as well as adults of E. serrulatus were infected with P. filicollis and all three stages, for 10 days at 20°C at least, supported an equal amount of procercoid development (Table I). By the 10th day all nauplii had metamorphosed into early copepodite stages.

4. Procercoid growth in cultured and non-cultured E. serrulatus.

From the measurements of the length and breadth (diameter) of the procercoids, their volumes were calculated assuming them to be cylinders. As shown in Fig. 7 the majority of procercoids, developed for 10 days in field copepods from the R. Kelvin had a volume considerably greater than 0.0001 cmm, while those developed for the same time in cultured copepods were usually less than 0.0001 cmm. The average 10 day procercoid in cultured and field copepods was 0.0000803 cmm and 0.000423 cmm respectively.

Figure 6

Procercoids (P) of Proteocephalus filicollis released after 25 days development from the haemocoel of the same Eucyclops serrulatus. One procercoid had an attached cercomer (C) but lacked suckers. The other procercoid had invaginate suckers (S) and had shed its cercomer.

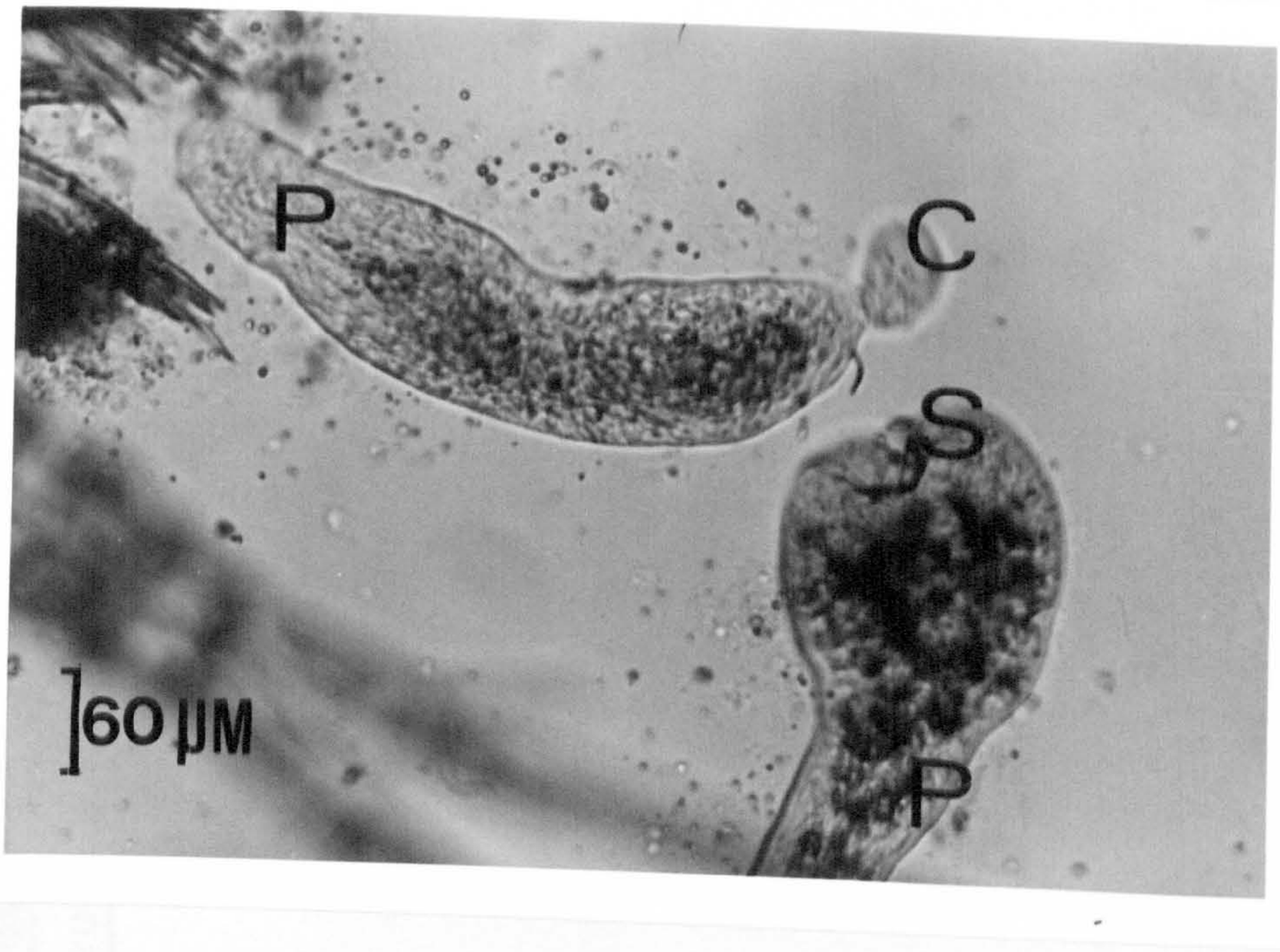

P
C
S
P
60 µM

Table I The growth of Proteocephalus filicollis procercoids in nauplii, copepodites and adults of Eucyclops serrulatus at 20°C

Time			Nauplii	Copepodites	Adults
5 days	Examined		2	5	5
	Infected		2	5	5
	No.of procercoids		2	11	10
	Procercoid size µm	mean	50x44	44x37	47x37
		range		33x24–66x44	37x22–51x44
10 days	Examined		3*	4	3
	Infected		3	4	3
	No.of procercoids		4	8	5
	Procercoid size µm	mean	78x43	65x55	70x56
		range	44x40–117x50	44x44–81x62	51x44–99x66

* By 10 days all the original nauplii had metamorphosed to copepodites

Fig 7 The volume of P.filicollis procercoids after 10 days development in Eucylops serrulatus from culture and from the field (R.Kelvin)

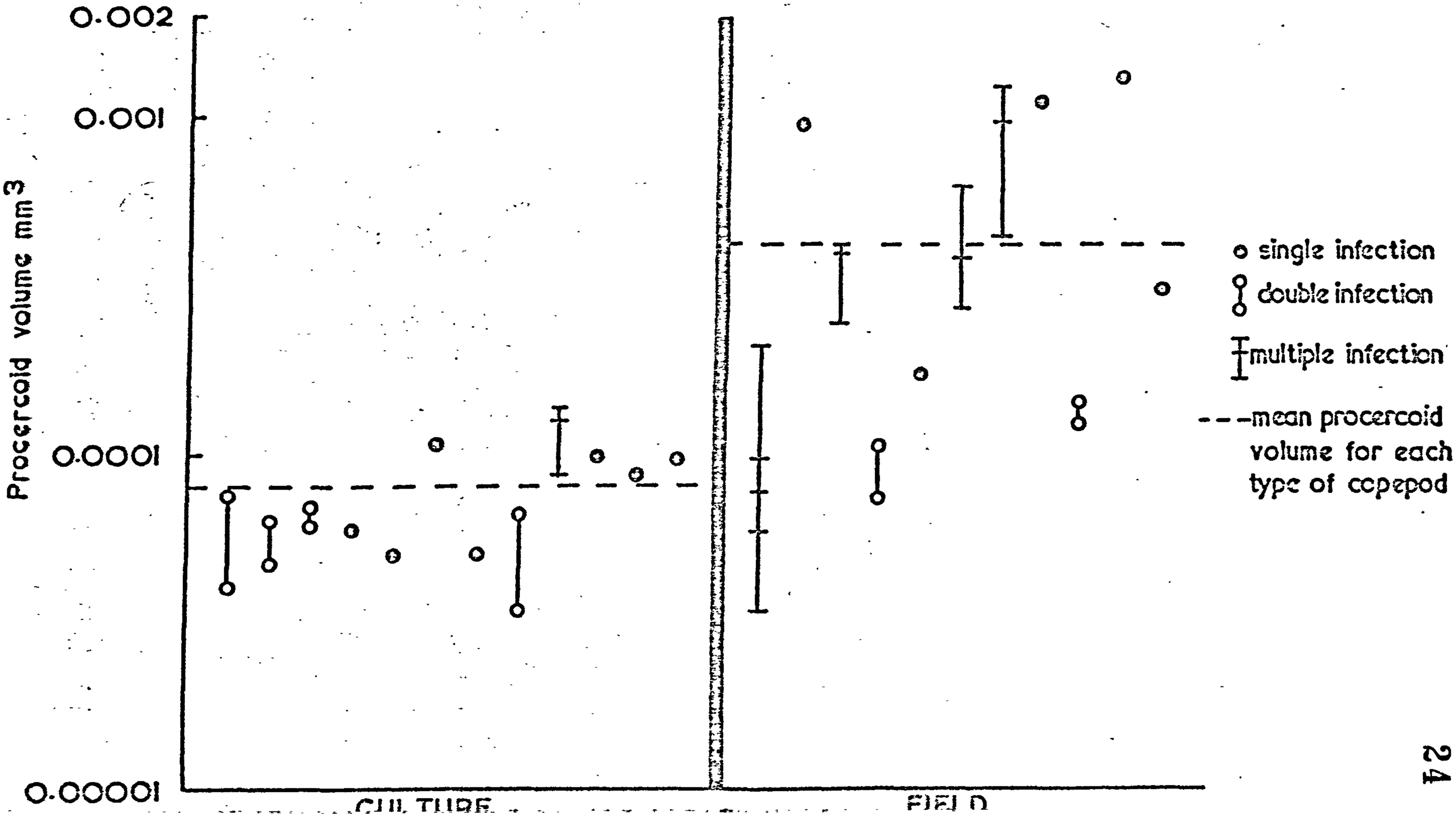


Fig. 8 indicates that field copepods are consistently considerably longer than cultured copepods.

5. Infection of sticklebacks with laboratory reared procercoids.

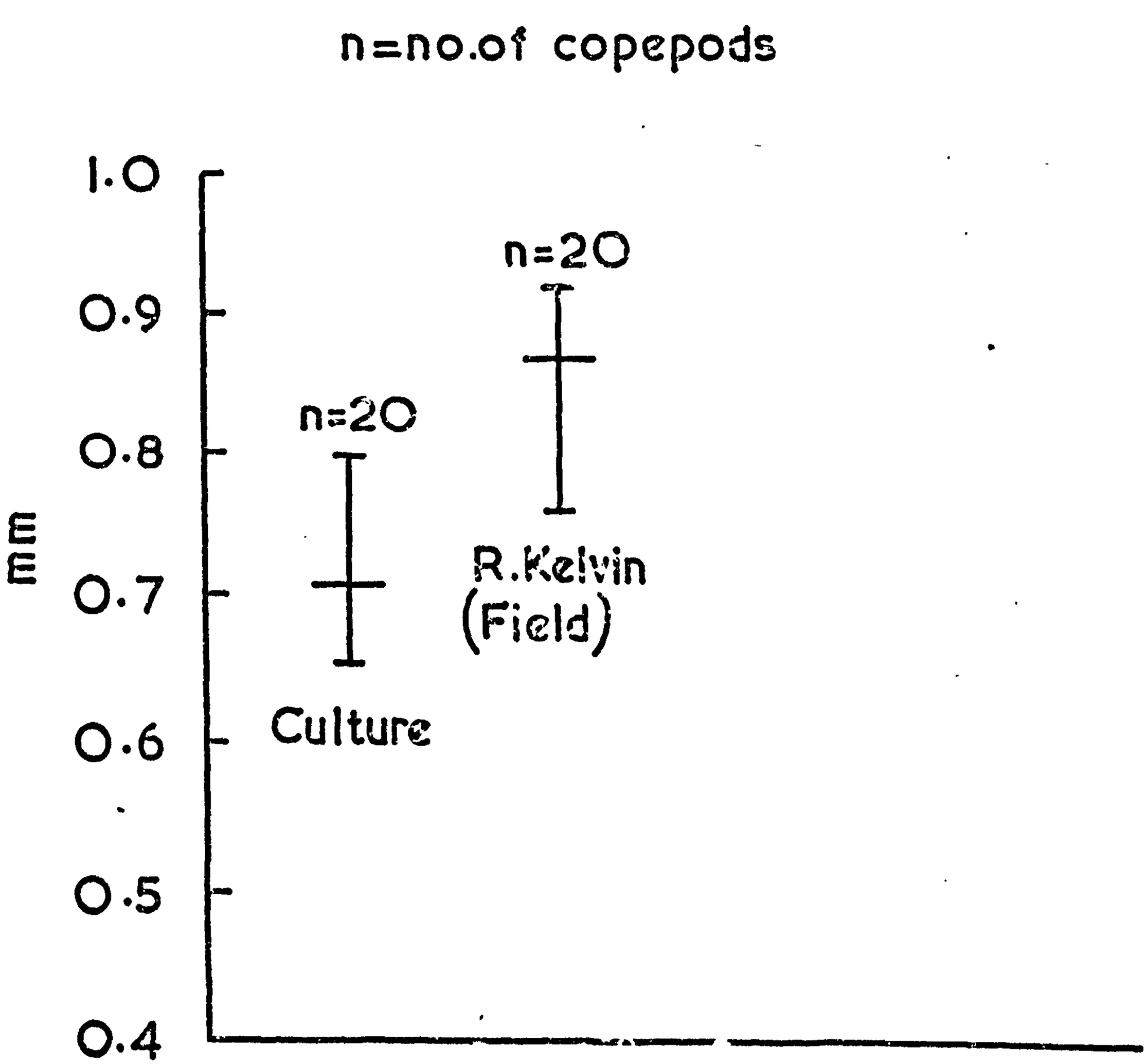
As shown in Table 2 of the 29 procercoids administered in copepods to the fish, only 5 (17.2%) were recovered 24 h later as young plerocercoids attached to the fish intestine. No day old plerocercoids were attached in the fish rectum. Day old plerocercoids ranged in length from 280 μm to 600 μm. All control fish proved to be free of P. filicollis infection.

6. Maintenance of P. filicollis in sticklebacks in the laboratory.

The incidence of P. filicollis in the Glasgow pond, as indicated in Table 3, from the 1st of July 1968 to the 2nd of August 1968 fell from 68% to 24.6% while the mean worm burden (i.e. the number of worms per infected fish) fell from 7.0 to 1.7, representing an 90.3% worm loss in 1 month (See also Section 3).

The incidence of Proteocephalus filicollis in stickle-backs collected from the same pond on the 11th July was 85.7%, while by the 8th August, after 28 days of laboratory

Fig 8 The distance between the anterior end of the cephalothorax and the posterior end of cultured and Field (R.Kelvin) copepods (E.serrulatus)



able 2 Infection of Gasterosteus aculeatus with Proteocephalus filicollis procercoids which had developed for 39 days at 20°C in E.serrulatus. Fish were examined 24h after copepod ingestion.

No.	Fish No.	No. of Copepods Fed	No. of Procercoids Fed	Worms recovered after 24h No.	Position	Length of worms
1	2	3	3			
2	2	3	0		Intestine	360,360,280 µm
3	2	3	0			
4	2	3	0			
5	1	4	0		Intestine	600 µm
6	2	4	1			
7	2	4	0			
8	1	3	0		Intestine	480µm
9	1	2	1			

Table 3 Survival of Proteocephalus filicollis in Gasterosteus aculeatus in the laboratory at 20°C and in the field 17–20 °C

	Field sample 1st July	Field sample 2nd Aug	Day O Check sample 11th July	Day 15 Laboratory maintenance 26th July	Day 20 Laboratory maintenance 31st July	Day 28 Laboratory maintenance 8th Aug
Number examined	60	81	7	10	5	10
of fish infected	41	20	6	5	3	1
Infection %	68	25	86	50%	60%	10%
No.of worms	289	34	35	13	4	1
Mean worm burden	7.0	1.7	5.8	2.6	1.3	

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of fish infected	41	20	6	5	3	1
Infection %	68	25	86	50%	60%	10%
No.of worms	289	34	35	13	4	1
Mean worm burden	7.0	1.7	5.8	2.6	1.3	

maintenance at 20°C the incidence had fallen to 10%. The mean worm burden also fell over the same period. From the 11th to the 26th July the mean worm burden was halved.

The number and condition of the wormsrecovered from the fish during the period of laboratory maintenance is indicated in Table 4.

7. <u>The transfer of</u> P. filicollis <u>from one stickleback to another</u>.

The 12 control fish which did not ingest infected stickleback intestines, were negative. 10 of the 18 experimental fish, shown in Table 5, were infected with <u>P. filicollis</u>. Both plerocercoids and strobilate worms had established themselves in new hosts. The gravid worm found after two days in a recipient host was then successfully transferred, in the same way, to another new host. The next transfer attempt, however, failed.

Table 4 The number and length of plerocercoids, immature (I), mature (M) and gravid (G) *Proteocephalus filicollis* recovered from Sticklebacks *Gasterosteus aculeatus* infected in the field but maintained in the laboratory.

	No.of worms	No.of plerocercoids	Average length and range of plero'coids (mm)	No.of strobilate worms	Average length and range of strobilate worms (mm)	Details of strobilate worms		
						length (mm)	seg.no.	conc
0 Day	35	34	0.77 (0.36–1.92)	1	216 (4segs)	2.1	4	I
15 Days	13	4	1.8 (1.2–3.0)	9	6.0 (1.2–9.6)	9.6	13	G
						9.2	14	M
						7.2	9	M
						5.6	9	I
						5.4	10	I
						5.2	8	I
						4.6	8	I
						1.2	3	I
						?	3	I
20 Days	4	0	——	4	2.7 (2.6–2.9)	2.9	5	I
						2.8	3	I
						2.6	2	I
28 Days	1	1	1.34	0	——	——		

Table 5 Infection of sticklebacks (G.aculeatus) with P.filicollis. The intestines of Sticklebacks containing established infections were fed to uninfected sticklebacks

Days post gut ingestion	No. of fish exam'd	No. of fish infected	No. of worms	Number of plerocercoids	Number of strobilate worms
2	2	1	1	0	1 gravid *
5	12	7	9	7	2 mature
9	4	2	3	2	1 immature
CONTROL FISH	12	0			

* this worm was then transferred successfully using the same technique to another fish. The next transfer failed.

DISCUSSION

(a) Egg structure

Meggitt (1914), in his pioneer study of Proteocephalus filicollis, noted that after egg release, the oncosphere passed through a pore in the embryophore and came to lie within the confines of the outer membrane. This phenomenon was not observed in the present study, although it has been reported for other proteocephalids (Essex 1928, Hunter 1929, Wagner 1954, Jarecka & Doby 1965). The statement of Freze (1965) that 'the oncosphere usually tears through the internal membrane while still in the water, and penetrates into the cavity between the internal and external egg membranes' is difficult to accept considering the contrary findings of Hunter (1928), Freeman (1964), Fischer (1967) and Wagner (1954). It seems unlikely, as pointed out by Hopkins (1959), that the movement of the oncosphere of Proteocephalus pinguis within the outer membrane, as suggested by Hunter (1929), would attract a copepod since the behaviour of E. serrulatus in the presence of P. filicollis eggs suggests that the senses of touch and probably taste are much more important than sight in the discovery and subsequent ingestion of eggs. In this connection Mueller (1965) states that copepods detect the

active coracidia of Spirometra mansonoides by 'bumping into them' rather than by seeing them.

(b) Egg longevity

Under laboratory conditions Proteocephalus filicollis eggs do not survive for particularly long even at low temperatures (Fig. 3). Since every effort was made to maintain the eggs under favorable conditions it is felt that the results probably reflect reasonably accurately egg longevity under natural conditions. Indeed when one considers the multitude of hazards eggs face in nature, e.g. predation and silt, it seems likely that eggs in the laboratory may survive longer than those in the field. Since eggs very soon lost their infectivity to copepods at 25°C, and yet retained it for over two weeks at 20°C, it would seem that the upper temperature limit for Proteocephalus filicollis transmission lies between 20°C and 25°C. This assumption is strengthened by the fact that procercoid development of P. filicollis in E. serrulatus ceases after 7 to 8 days at 25°C (Gemmell unpublished results). At the other extreme, the inability of P. filicollis eggs to survive longer than 5 to 6 weeks at 4°C, indicates that this worm is incapable of overwintering as an egg.

Likewise eggs of Proteocephalus tumidocollus cannot overwinter, remaining alive for only 30 days at temperatures ranging from 0°C to 10°C (Wagner, 1954). Thus it would appear that some, if not all proteocephalid tapeworms of fish, are completely dependant on their hosts for survival through the winter period. It has been suggested that both P. filicollis (see Sections 2 &3) and a proteocephalid infecting Coregonus lavaretus in Loch Lomond (see Section 5) overwinter in both the copepod and definitive fish host.

(c) The procercoid

Procercoid development of Proteocephalus filicollis was described in Eucyclops serrulatus s.s. (Fischer) by Meggitt (1914) who referred to the copepod by its older name Cyclops varius (Lilljeborg). While 39 day procercoids were shown to be infective to sticklebacks (Table 4), some 25 and 30 day old worms were possibly also infective. The variation in the rate of procercoid growth renders it impossible to be specific as to when P. filicollis procercoids become infective.

Although the proteocephalid cercomer is rudimentary (Ginetsinskaya 1961) and not present in some species

(Hunter 1928,1929), it was observed frequently in the present study. Hooks were never found on the cercomer, they remained loosely scattered in the tissue of the developing procercoid. Once formed the proteocephalid cercomer is quickly shed (Freze 1965) and this fact may explain why cercomer formation by P. filicollis procercoids was not noted by Meggitt (1914).

Contrary to the findings in the present study Meggitt (1914) states that nauplii of E. serrulatus were never infected experimentally with P. filicollis. Although few measurements were taken, it would seem that, at least during the first 10 days of procercoid development, the stage of copepod host has little effect on worm growth rate. Ewars (1936) noted that stage I nauplii of E. serrulatus require 6 to 7 days at 18°C to become early copepodites. The fact that all nauplii in this study were copepodites by 10 days indicates that the presence of developing procercoids did not markedly retard the development of the young copepods. However, developing procercoids of Spirometra mansonoides retard and sterilize their host copepod Cyclops vernalis (Mueller 1965). Meggitt (1914) stated that 'the presence of the larva is fatal to the Cyclops causing its starvation.'

Although Meggitt (1914) recorded that oncospheres of P. filicollis took 'usually a week' to penetrate the copepod gut, penetration was observed (Fig. 3) within a few hours of egg ingestion as in other proteocephalids (Wagner 1954, Thomas 1931 and others). The original observation of Meggitt thus seems doubtful.

It is quite clear that the copepod, E. serrulatus, after 8 months of laboratory culture was considerably smaller (Fig. 8) and a less suitable host for procercoid development of P. filicollis (Fig. 7), than specimens of the same species collected fresh from the field. This reduced ability of cultured copepods to allow normal procercoid development cannot, as in Mueller's (1965) studies of Spirometra mansonoides in Cyclops vernalis, be attributed to selection of resistant strains, since the copepod breeding tanks were never exposed to infection.

Cultured copepods also lacked the oil globules of field specimens and were extremely delicate being easily damaged by coverslip pressure unlike the more robust copepods from the field. These findings question the usefulness of long term laboratory culture of copepods in the study of tapeworm development. As pointed out by Orr & Hopkins (1969) the whole problem of laboratory

propagation of copepods should be investigated in detail.

(d) The plerocercoid and adult

Unlike proteocephalid procercoids, little is known about the growth and development of plerocercoids and adults in the definitive fish host. Ecological studies of proteocephalids in fish (Hopkins 1959, Kennedy & Hine 1969, and Sections 2, 3 & 5) indicate the seasons of the year in which worms grow and mature but tell little of the rate of growth and development at specific temperatures. To determine the latter, however, one must rely on controlled laboratory studies which would require that the proteocephalid infection could be maintained in fish under laboratory conditions. The tremendous loss of immature worms from sticklebacks maintained in the laboratory for 28 days was no artifact, since a similar worm loss occurred in the field (Table 3). Similarly Kennedy & Hine (1969) experienced difficulty in maintaining immature Proteocephalus torulosus in dace Leuciscus leuciscus under laboratory conditions. Ecological studies of P. filicollis in sticklebacks, Gasterosteus aculeatus (Hopkins 1959, and Sections 2 & 3), of P. torulosus in dace Leuciscus leuciscus (Kennedy & Hine 1969), and Caryophyllaeus laticeps in dace

(Kennedy 1969) indicate that the loss of immature worms from their definitive fish host in nature is the rule rather than the exception. Thus the problem of maintaining proteocephalid worms in fish for study of later stages of growth and development requires the initial infection and maintenance of large numbers of fish.

Observations on the early development of P. filicollis in the fish at 20°C (Table 4), showed that Day 0 plerocercoids with a mean length of 0.77 mm grew to a mean of 1.8 mm in 15 days. Fischer (1968) found that newly acquired plerocercoids of Proteocephalus fluviatilis tripled their length in 13 days at 18°C.

The ability of Proteocephalus filicollis to withstand transfer from one stickleback to another (Table 5) may be of use in the investigation of worm growth and development in the fish host. Thus the length and condition of a worm could be noted before and after a period of development under controlled conditions in a new host. The results accumulated from a number of experiments of this type would produce a complete picture of proteocephalid worm development in fish maintained at various temperatures under various conditions. The technique of Willemse (1968), whereby worms were introduced into the intestine of recipient fish per anus, might prove useful in such a

study.

This ability of *P. filicollis* to establish itself in a new host is ecologically insignificant since there is little evidence (Hynes 1951) that sticklebacks are canibalistic. Fischer (1968) has shown that *Proteocephalus fluviatilis* plerocercoids and strobilate worms can, once removed from a bass, *Micropteous dolomieui* be fed to, and reestablish in, another bass. In this case the ability of the worms to reestablish in a new host is of ecological significance since the heavy infections of *P. fluviatilis* in large bass, which seldom eat copepods, can only be explained by their predation on bass fry (Fischer 1968).

While only a few of the procercoids fed to sticklebacks succeeded in becoming established as plerocercoids (Table 2) it is clear that the favoured site of establishment was the intestine, no worms being found in the rectum. Hopkins (1959) and Willemse (1968) found that virtually all plerocercoids of *P. filicollis* were, in their field studies, attached in the stickleback rectum, while it has been argued elsewhere (see Sections 2 &3) that plerocercoid attachment probably occurs in both regions and that plerocercoids can migrate from the intestine to the rectum and *vice versa*. This whole problem of the preferred site

of plerocercoid establishment requires further investigation.

(e) The maintenance of P. filicollis in the laboratory.

The complete life cycle of Schistocephalus solidus can be established in the laboratory (Orr & Hopkins 1969). For many reasons, however, the maintenance of P. filicollis is a much more difficult task. Firstly procercoid development in the copepod host takes 25 to 40 days, two to three times longer than that of S. solidus. Secondly immature worms are extremely difficult to maintain in the fish intestine. Thirdly each gravid worm produced relatively few eggs compared with the tens of thousands of eggs produced by S. solidus adults. P. filicollis is also an unsuitable tapeworm for laboratory experimental purposes because unlike S. solidus plerocercoids in sticklebacks, the supply of both immature and gravid P. filicollis from the field is extremely variable, worms being virtually absent from all fish in some months (Hopkins 1959 and Sections 2 & 3).

SUMMARY

(1) Eggs of Proteocephalus filicollis remained infective to copepods for 12, 14, 14, and 44 days at 20°C, 15°C, 10°C and 4°C respectively. Eggs after 3 days at 25°C were non-infective.

(2) Procercoids of Proteocephalus filicollis after 39 days of development at 20°C in the copepod Eucyclops serrulatus s.s. proved infective to three-spined sticklebacks Gasterosteus aculeatus. Cercomer formation by the procercoid occurred between the 15th and 25th days. Nauplii, copepodites, and adults of Eucyclops serrulatus s.s. were equally susceptible to infection with P. filicollis.

(3) Due to loss of worms from the fish gut, an attempt to maintain an established P. filicollis infection in sticklebacks in the laboratory failed. Both immature and gravid P. filicollis were, however, transferred successfully from one stickleback to another.

(4) Proteocephalus filicollis was found to be a difficult tapeworm to maintain in the laboratory.

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SECTION 2

Investigations into the biology of *Proteocephalus filicollis* (Rud. 1810) a cestode parasite of the three-spined stickleback, *Gasterosteus aculeatus* (L.)

(2) Incidence and maturation in a canal in Glasgow.

(with 12 figures in the text)

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INTRODUCTION

Proteocephalus filicollis, a cestode parasite of the three-spined stickleback Gasterosteus aculeatus, was shown by Hopkins (1959) and Willemse (1968) to possess a distinct seasonal incidence and maturation cycle in a Lanarkshire pond and in the canals of the Netherlands. An initial investigation of two stickleback sites, a canal and a small pond in Glasgow, indicated that, if maturation and incidence cycles of P. filicollis existed in these sites, they were very different from the cycle described by Hopkins (1959) and Willemse (1968). A two year study of P. filicollis in these two Glasgow sites was therefore undertaken.

Both the plerocercoid and adult P. filicollis are found in the intestine of Gasterosteus aculeatus. The copepod Eucyclops serrulatus (Fischer) is suitable for procercoid development (Section 1). There is no second intermediate host, the stickleback acquiring the parasite by eating infected copepods.

Briefly the seasonal cycle of maturation described by Hopkins (1959) and Willemse (1968) indicated worm maturation and egg release in early summer, while the worm population throughout the remainder of the year

consisted of immature worms, mainly plerocercoids. During the present studies, however, Chappell (1969) published evidence of a different type of cycle by P. filicollis in a Yorkshire pond. Although his samples were bimonthly instead of monthly, as in previous and the present studies, there is evidence from his results that mature and gravid worms occurred throughout the year. Chappell's findings would suggest that P. filicollis need not necessarily possess, in every site, the same type of incidence and maturation cycle as described by Hopkins (1959) and Willemse (1968).

The results of the two concurrent studies of the ecology of P. filicollis are presented in separate sections. The incidence and maturation of P. filicollis in the canal site is described and discussed in this section, while the survey based on the small pond is presented in Section 3 .

MATERIAL AND METHODS

1. The Forth and Clyde Canal Site

Proteocephalus filicollis was found as a natural infection of *Gasterosteus aculeatus* inhabiting the disused Forth and Clyde Canal in N.W. Glasgow. The sampling site was located between two permanently closed lock gates 180 yards apart. Water flowed continuously over the gates resulting in a slow flow of water through the site. The shallow banks are densely weeded; most fish were caught amongst the weed. Little variation in the canal water level occurred. A great variety of invertebrates were noted, and perch, *Perca fluviatilus*, and roach *Rutilus rutilus*, were caught as well as three-spined sticklebacks.

2. Collection of fish

Approximately 60 fish were caught each month from September 1967 to December 1969 with a 4' beam trawl. Winter ice cover necessitated ice breaking and clearing before fishing. Difficulty in catching fish was experienced in winter resulting in poor samples being obtained in some months. As each sample was obtained, the temperature of the canal water at a depth of 15 cm was noted. The fish were taken alive to the laboratory and

kept in running water tanks at ± 20° or that recorded in the canal.

3. Examination of fish

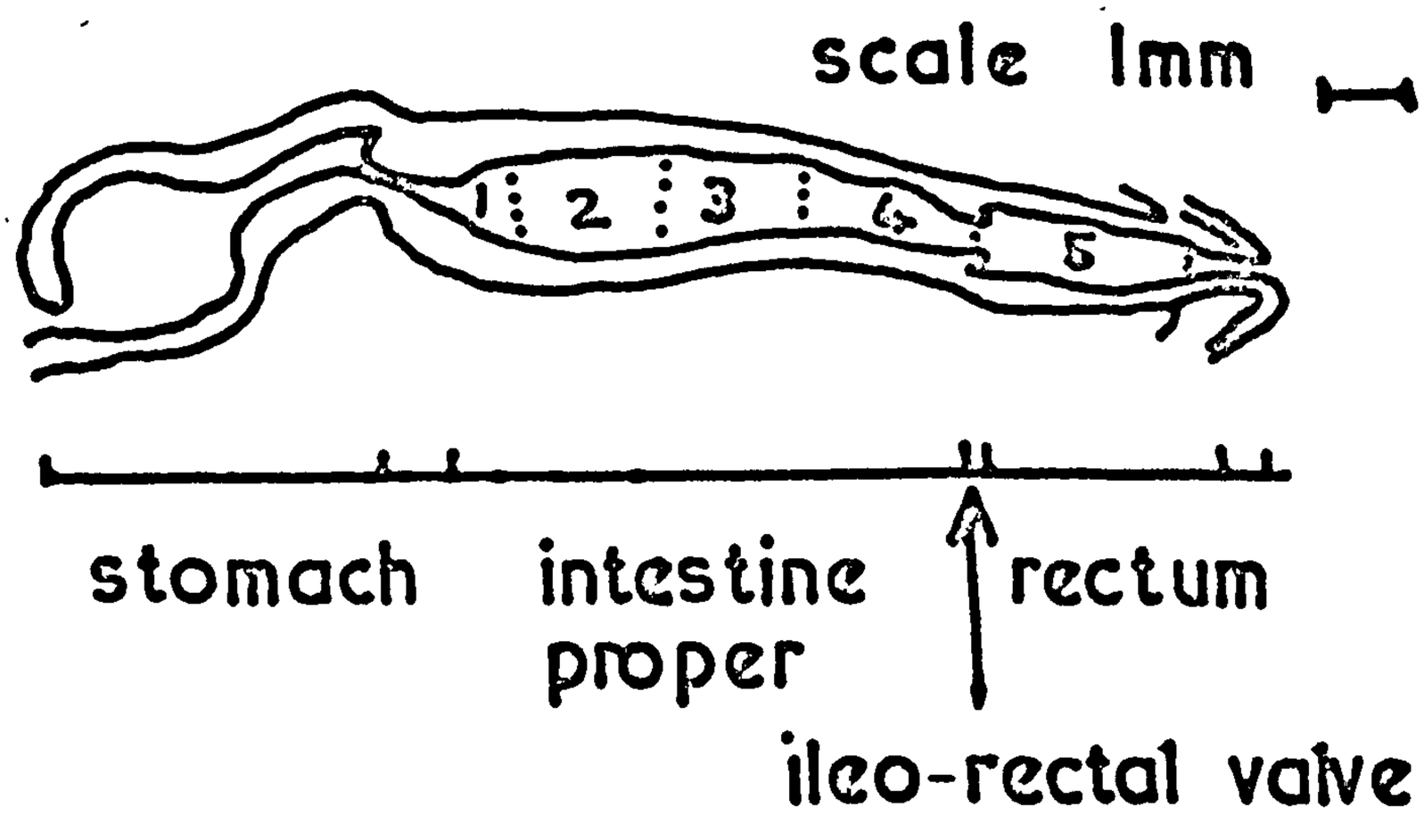
All fish were examined within 24 h of capture. Fish were killed by pithing and the abdomen opened ventrally with a cut extending back to the anus. The intestine, from stomach to rectum inclusive, was removed with forceps, placed on a 3 in. by 2 in. slide and examined under light pressure from a covering 2 in. by 1 in. slide, using transmitted light and X25 magnification. As shown in Fig.1, the gut was visually divided into five regions. Having noted the region to which the scolex of each worm present was attached, increased pressure was applied to the covering slide to expel both the worms and the gut contents from the anus. During the latter half of the survey a qualitative inspection of the gut contents was made.

4. Examination of worms

The numbers of gravid, mature and immature segments of each worm were counted. Segments full of eggs characterised segments classed as gravid, while well formed testes and ovaries accompanied by prominent vitellaria distinguished mature segments. Both strobilate worms and plerocercoids were transferred individually to watch glasses containing water at 4°C, allowed to relax until dead, and the length measured with a micrometer eyepiece.

Figure 1

Semi-diagramatic longitudinal section of the alimentary canal of Gasterosteus aculeatus (adapted from Hale 1965). The gut posterior to the stomach has been divided into 5 regions; 1. the pyloric region, 2. the anterior intestine, 3. the mid-intestine, 4. the posterior intestine and 5. the rectum.



RESULTS

Details of the numbers of fish caught, the numbers infected, the numbers of worms found, the incidence and mean worm burden each month are presented, along with the monthly temperature records in Table I.

1. Incidence and mean worm burden

The incidence was low in September and October 1967, considerably higher from November 1967 to April 1968, before falling in May 1968.(Fig.2)The incidence was higher in June and July 1968 reaching a peak in August 1968.The incidence then fell remaining low from October to December 1968 and then rose during the months January to April 1969. The incidence was low in May and June 1969, and then fell to low levels in November 1969.

The mean worm burden (i.e. the number of worms per infected fish) of adjacent months have been meaned (Fig 3) to smooth out chance variation (Table I). The mean worm burden over the survey was 1.97. The burden was low in September and October 1967, somewhat higher from November/December 1967 to September/October 1968. The worm burden was low in November/December 1968 and January/February 1969. Although relatively high in March/April 1969, the mean worm burden fell gradually throughout the remainder

Table I The incidence and mean worm burden of Proteocephalus filicollis in Gasterosteus aculeatus from Septembe 1967 to December 1969 in the Forth and Clyde Canal in Glasgow, together with the monthly temperature records.

MONTH AND YEAR	NUMBER EXAMINED	OF FISH INFECTED	INFECTION %	NUMBER OF WORMS	MEAN WORM BURDEN	TEMPERATURE °C
1967						
SEPT.	44	6	13·6	8	1·3	10·0
OCT.	83	11	13·2	16	1·4	10·0
NOV.	55	19	35·0	42	2·2	6·0
DEC.	30	13	43·3	36	2·7	5·0
1968						
JAN.	40	20	50·0	43	2·1	4·0
FEB.	31	15	48·3	31	2·0	4·0
MAR.	49	22	44·8	65	2·9	4·5
APR.	40	18	45·0	38	2·1	9·0
MAY	54	7	12·9	17	2·4	13·0
JUNE	60	22	36·6	54	2·4	21·0
JULY	71	26	36·6	52	2·0	15·5
AUG.	60	45	75·0	96	2·1	14·5
SEPT.	60	38	63·3	98	3·8	15·5
OCT.	56	18	32·1	33	1·8	12·0
NOV.	67	18	26·8	28	1·5	4·0
DEC.	61	18	29·0	31	1·7	4·5
1969						
JAN.	51	21	41·0	29	1·3	2·5
FEB.	62	29	46·7	62	2·1	3·0
MAR.	58	26	44·8	56	2·1	3·0
APR.	42	23	54·7	59	2·5	5·0
MAY	60	12	20·0	22	1·8	11·5
JUNE	61	10	16·3	21	2·1	15·5
JULY	60	13	21·6	19	1·4	17·0
AUG.	60	17	28·3	23	1·3	18·0
SEPT.	61	20	32·7	35	1·7	17·0
OCT.	60	13	21·6	18	1·3	10·5
NOV.	60	9	14·9	10	1·1	10·0
DEC.	60	18	30·0	23	1·2	4·0

Fig 2 The incidence of infection of Proteocephalus fillicollis in Gasterosteus aculeatus

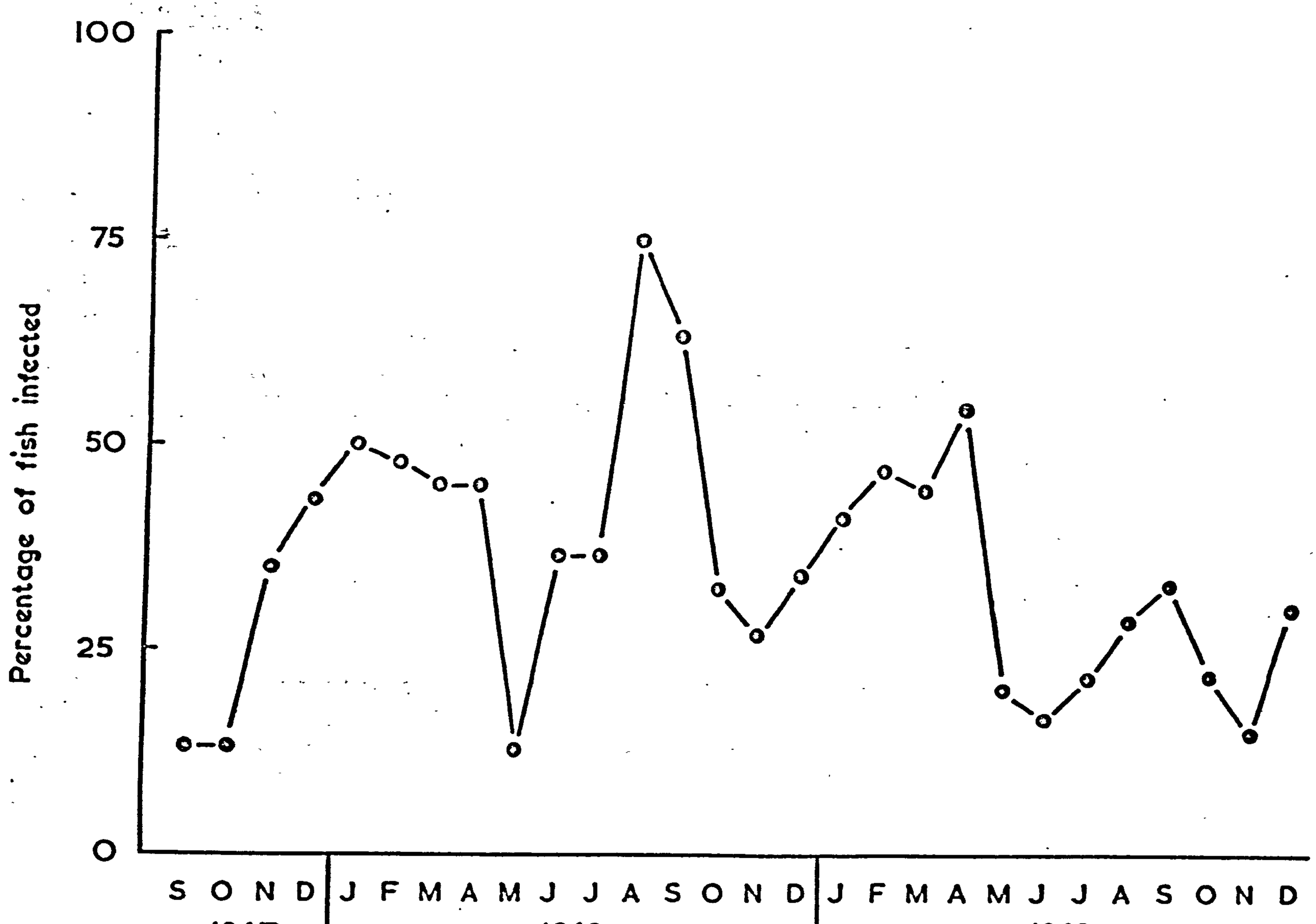
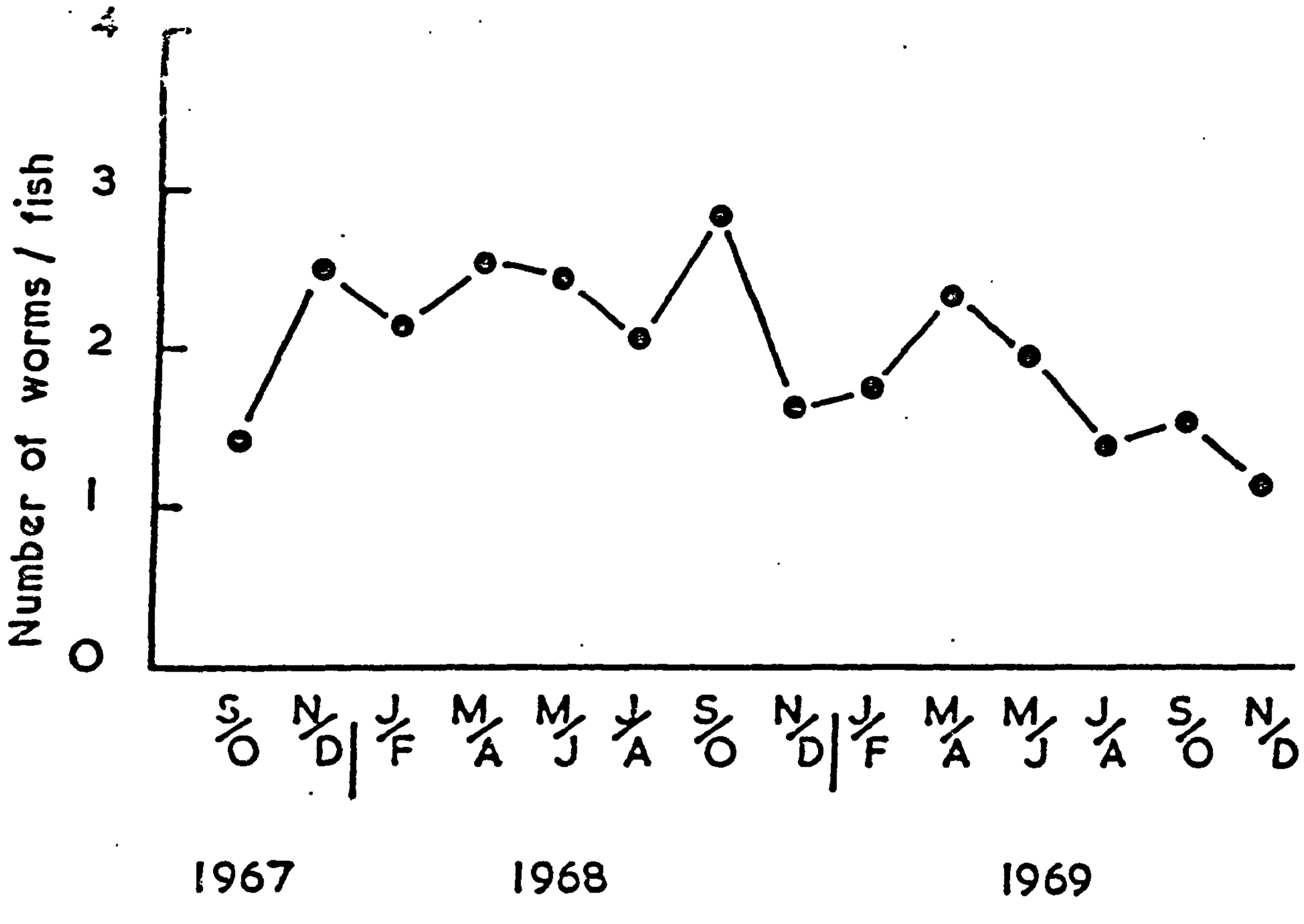


Fig 3 The mean worm burden of Proteocephalus filicollis in Gasterosteus aculeatus from September 1967 to December 1969



of 1969.

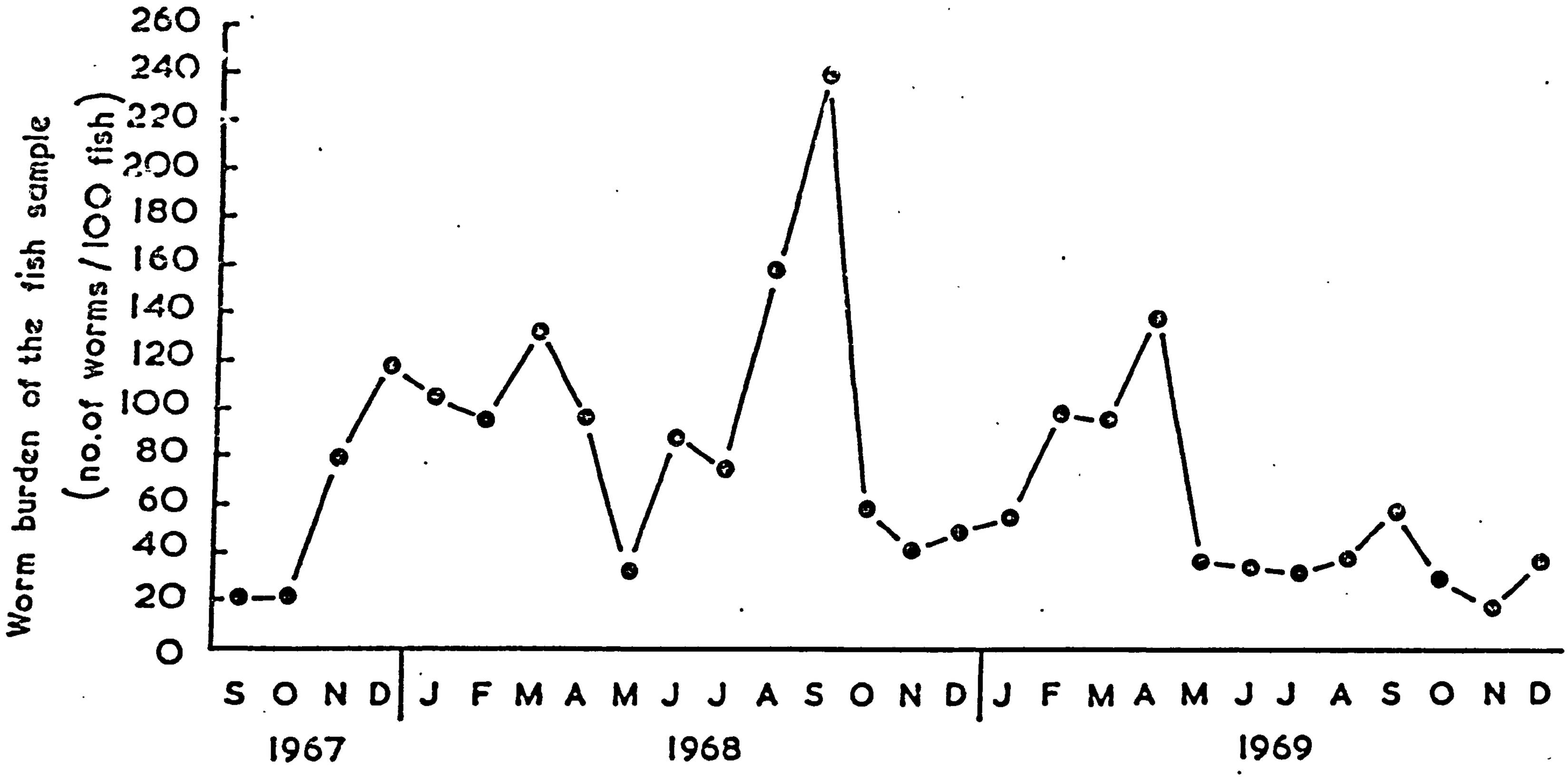
As the incidence and mean worm burden did not always rise and fall in unison (Table I) the product of the incidence and mean worm burden (the mean worm burden of the fish sample) is useful as it yields a clearer picture of the dynamics of the worm population.

2. <u>The worm burden of the fish sample</u>

The worm burden of the fish sample was low in September and October 1967 (Fig. 4), relatively high from November 1967 to April 1968, and fell in May 1968. The worm burden of the fish sample then rose reaching a peak in September 1968. The burden fell in October 1968 remaining low until January 1969. The burden was relatively high from February to April 1969, fell in May 1969 and remained low for the rest of the year.

The period May to August 1969 illustrates the usefulness of calculating the worm burden of the fish samples. The incidence and mean worm burden rose and fell out of phase resulting in a confused situation (Table 1). The virtually constant worm burden of the fish samples during the period, however, indicates that the individual variations in incidence and mean worm burden were of little significance.

Fig 4 The Proteocephalus filicollis worm burden of Gasterosteus aculeatus each month from September 1967 to December 1969



3. Worm maturation

Although relatively scarce in April and May of each year (see Fig. 5), plerocercoids made up a very considerable percentage of the worm population throughout the survey period. Strobilate non-gravid worms were particularly prevalent in the above months, and although present throughout the survey were scarce from August to December 1968 and from July to October 1969.

Gravid worms occurred every month except September and October 1967 and May 1969.

4. Worm position

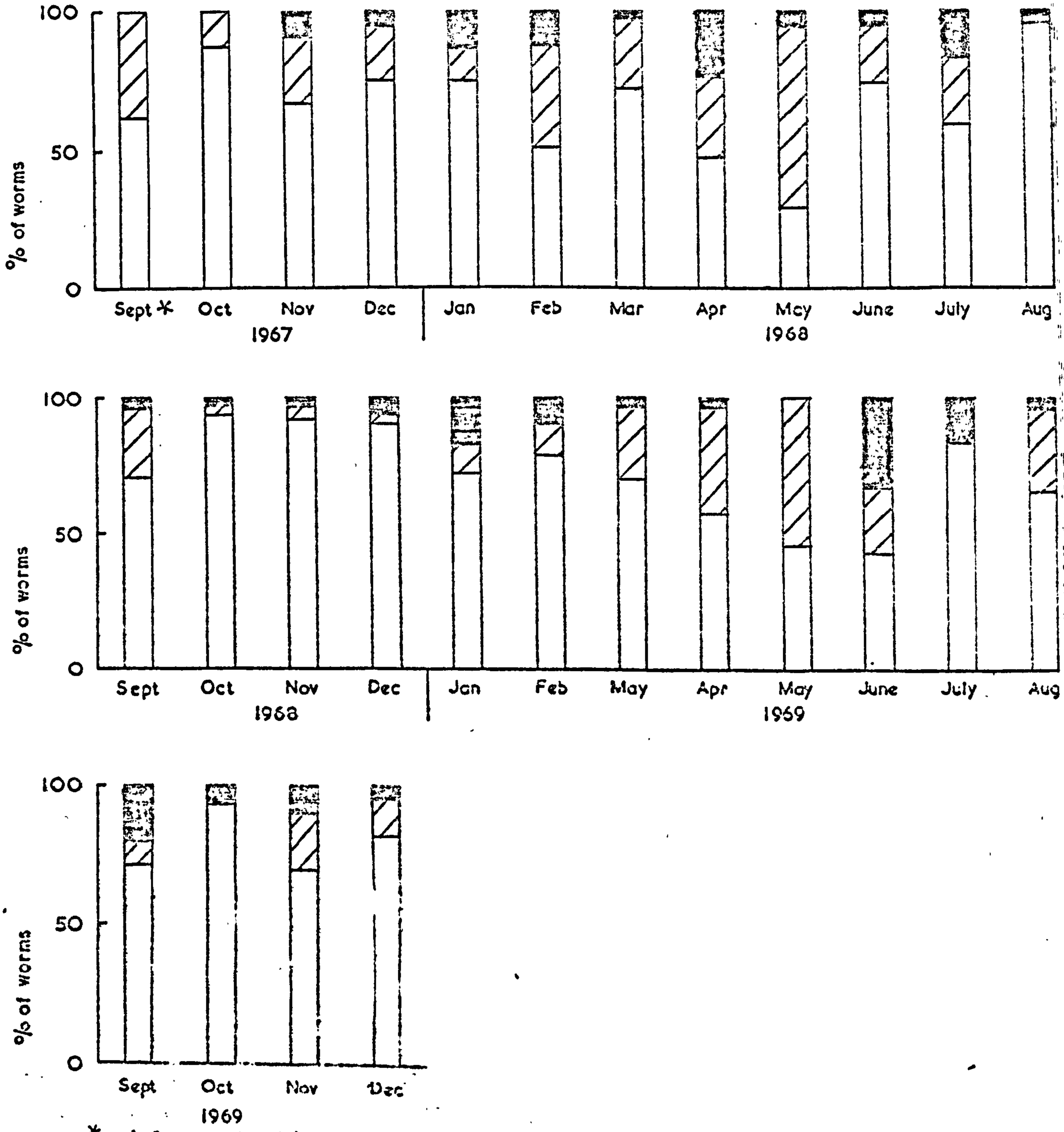
(a) The strobilate worm population

Of the strobilate worms 4 (1.5%) were attached in the rectum (Fig. 6). Of these 4 worms, 3 were gravid. Most strobilate worms were attached in the anterior and mid-intestine. No seasonal variation in the distribution of strobilate worms in the intestine occurred.

Gravid worms occurred in all regions, over 60% were found in the two anterior regions combined. Of the worms attached in the pyloric region 72% were gravid.

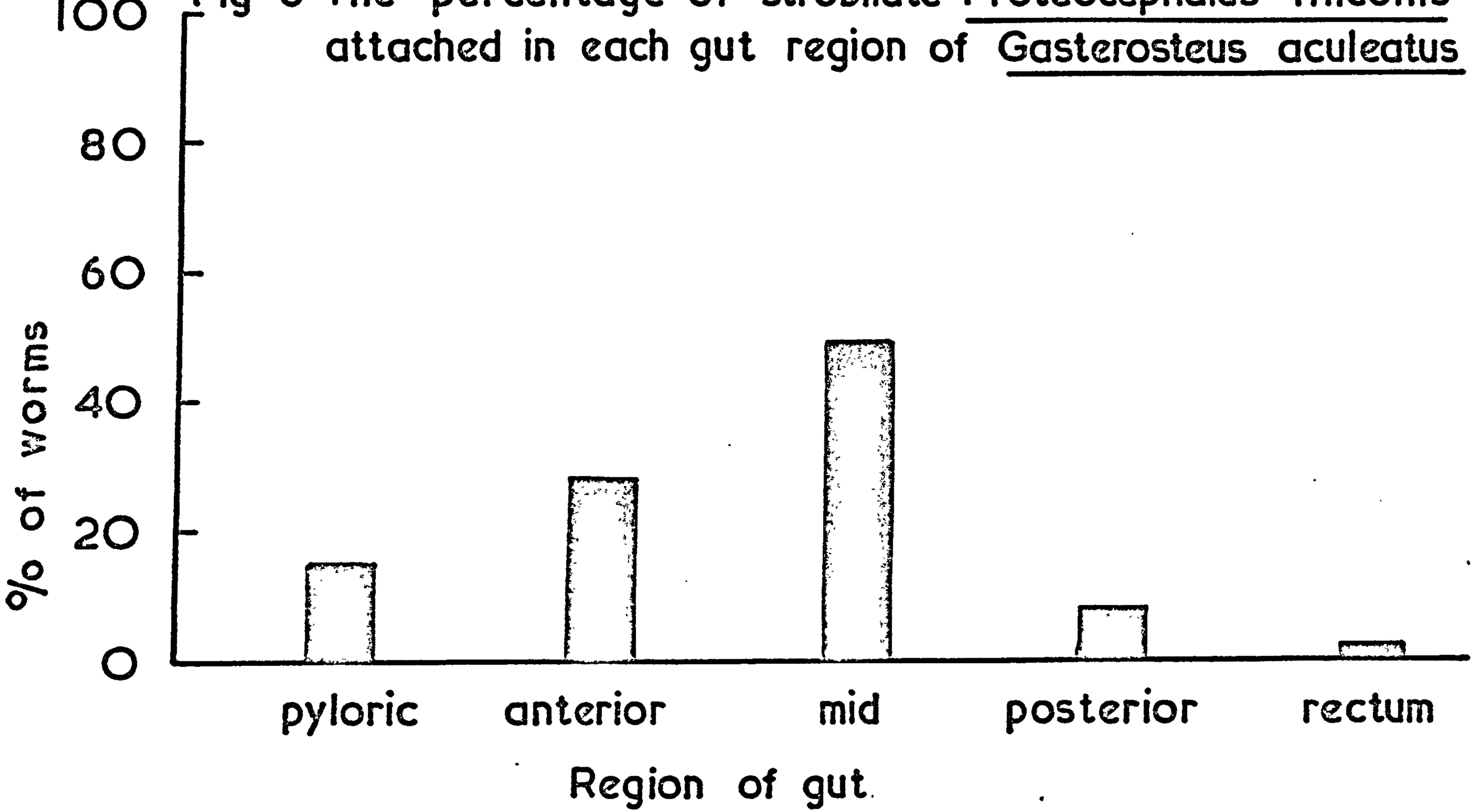
(b) Seasonal distribution of plerocercoids between the rectum and the intestine anterior to the ileo-rectal valve.

Fig 5 The percentage of plerocercoids (clear), strobilate non-gravid (crosshatched) and gravid (black) *Proteocephalus filicollis* in *Gasterosteus aculeatus*



* only 8 worms found in the Sept 1969 sample

Fig 6 The percentage of strobilate Proteocephalus filicollis attached in each gut region of Gasterosteus aculeatus



Plerocercoids were concentrated in the rectum in September and October 1967, from August to December 1968, and from September to December 1969 (Fig. 7). From February to July 1968, and from March to August 1969 plerocercoids were concentrated anterior to the ileo-rectal valve. A transitional period between plerocercoid concentration in the rectum and the 4 anterior intestine regions occurred from November 1967 to January 1968, and in January and February 1969. The May 1968 distribution is of doubtful significance as it is based on very few plerocercoids.

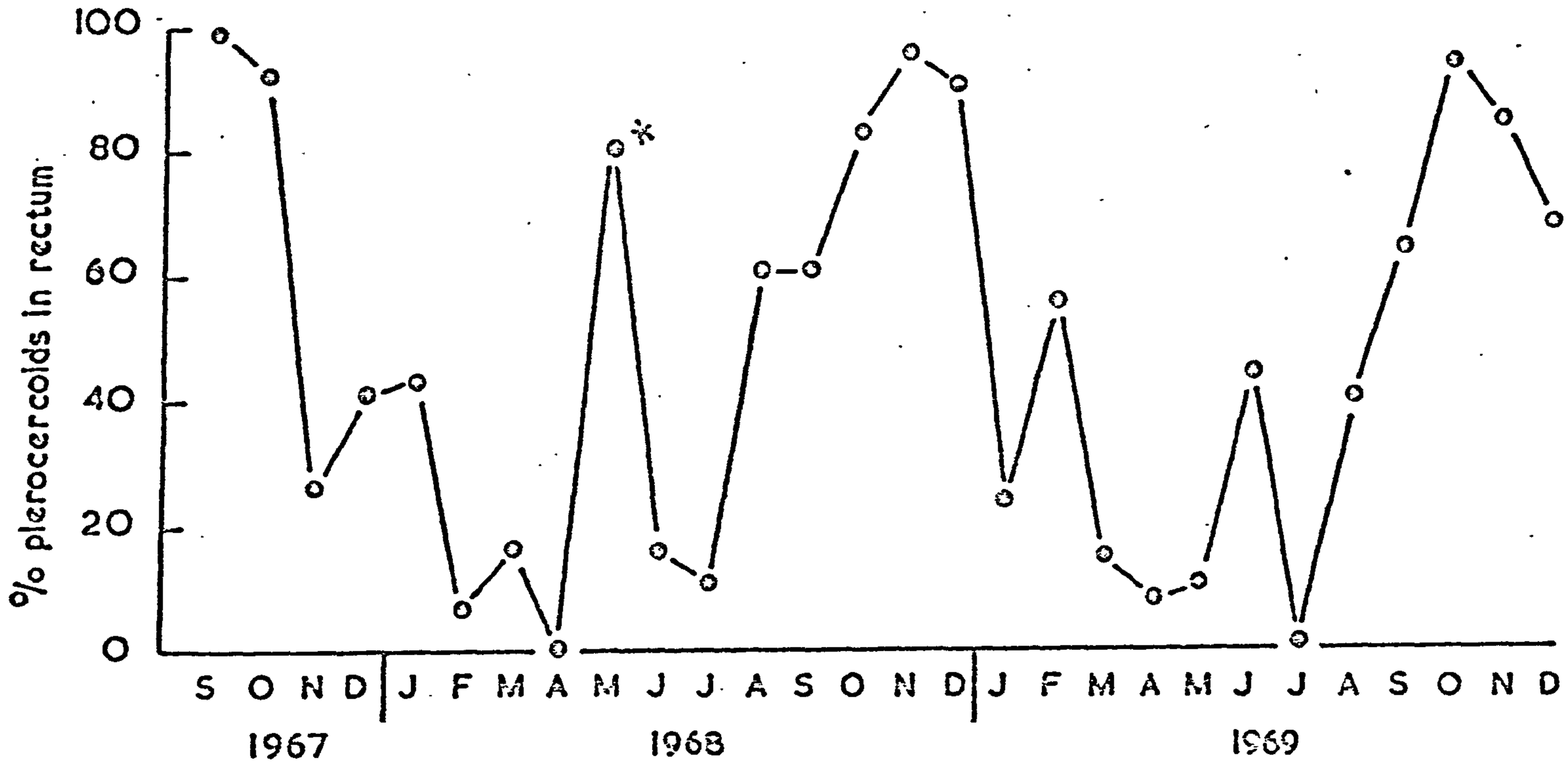
5. Worm size, segmentation and maturity

(a) Strobilate worms

As indicated in Fig. 8 gravid worms were generally longer than non-gravid worms. Gravid and non-gravid worms ranged in length between 6 and 36.8 mm, and 2 and 17.8 mm respectively. Thus both non-gravid and gravid worms made up the total worm population in the 6 mm to 17.8 mm length range.

Gravid and non-gravid worms possessed between 13 and 41, and 3 and 36 proglottids respectively (Fig.9). Thus worms with between 13 and 36 segments inclusive were either gravid or non-gravid, the more segmented worms being more likely to be gravid.

Fig 7 The percentage of plerocercoids of Proteocephalus filicollis attached in the rectum of Gasterosteus aculeatus from September 1967 to December 1969



* only 5 plerocercoids found

Fig 8 The relationship between maturity and length of strobilate Proteocephalus filicollis. The crosshatched area shows the number of non-gravid worms. The black area shows the number of gravid worms.

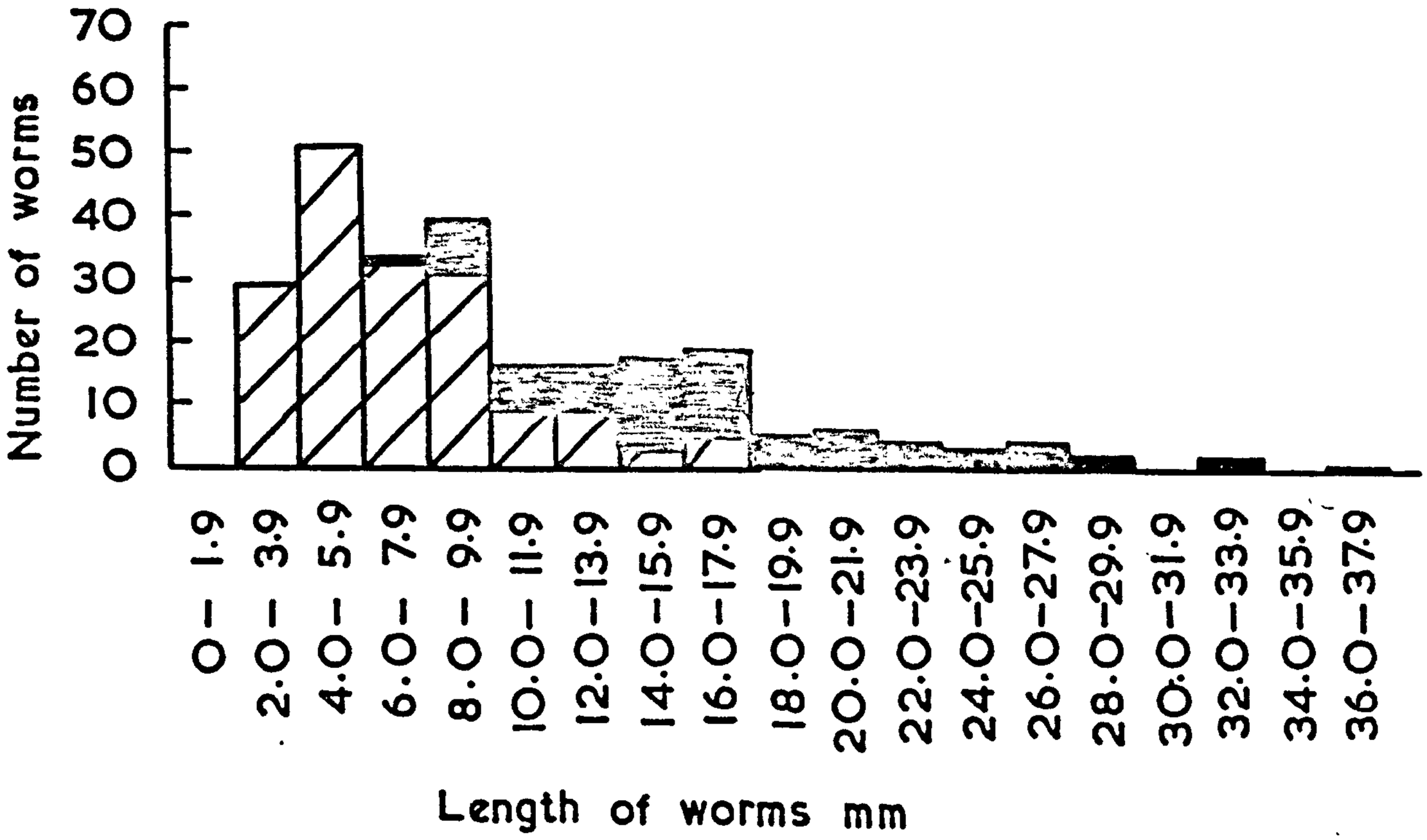
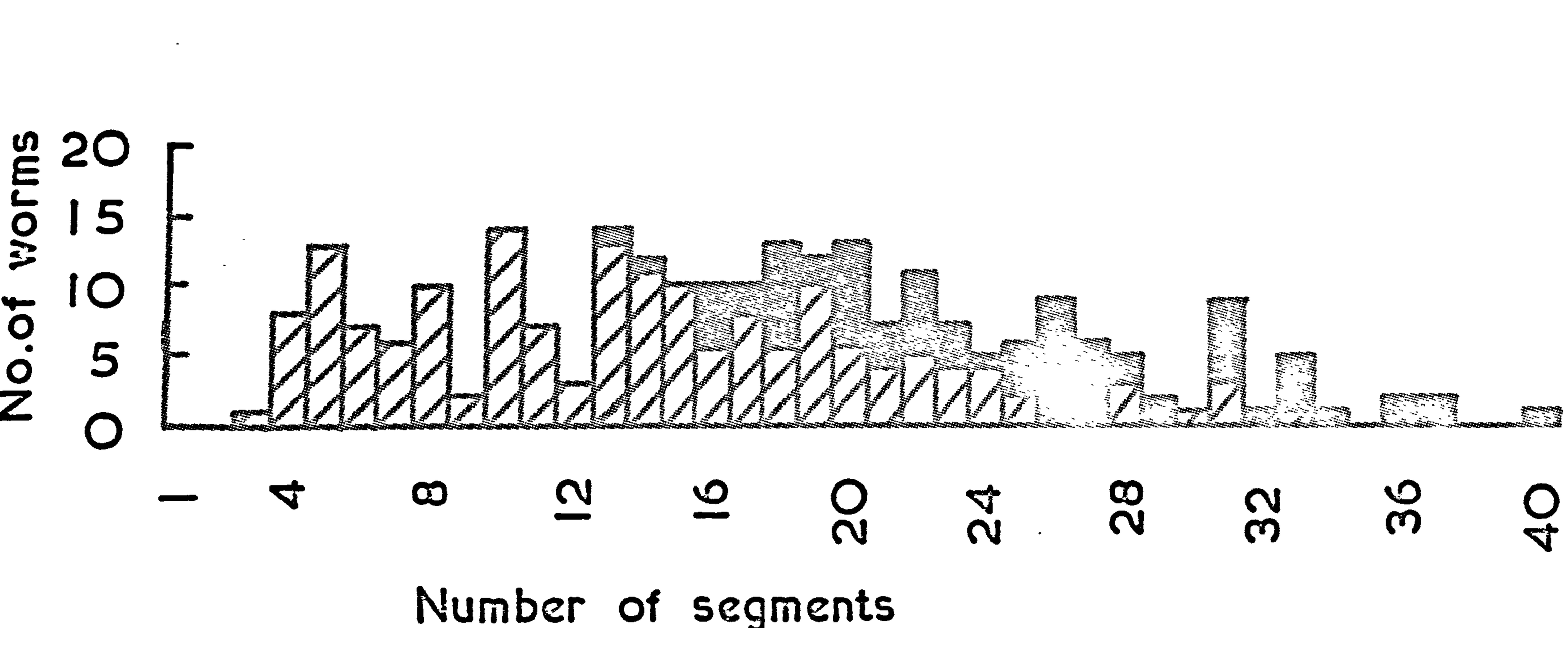


Fig 9 The relationship of number of segments of Proteocephalus filicollis and maturity. The crosshatched area shows the number of non-gravid worms. The black area shows the number of gravid worm



(b) Plerocercoid length

Plerocercoids ranged in length from 0.32 mm to 5.04 mm, mean 1.32 mm (702 measured). Plerocercoids less than 1 mm occurred each month, being most common from June to September 1968 and from July to September 1968 and from July to September 1969 when the mean length was around 1 mm or less (Fig. 10). The mean plerocercoid length throughout the rest of the year tended to be greater especially in late winter.

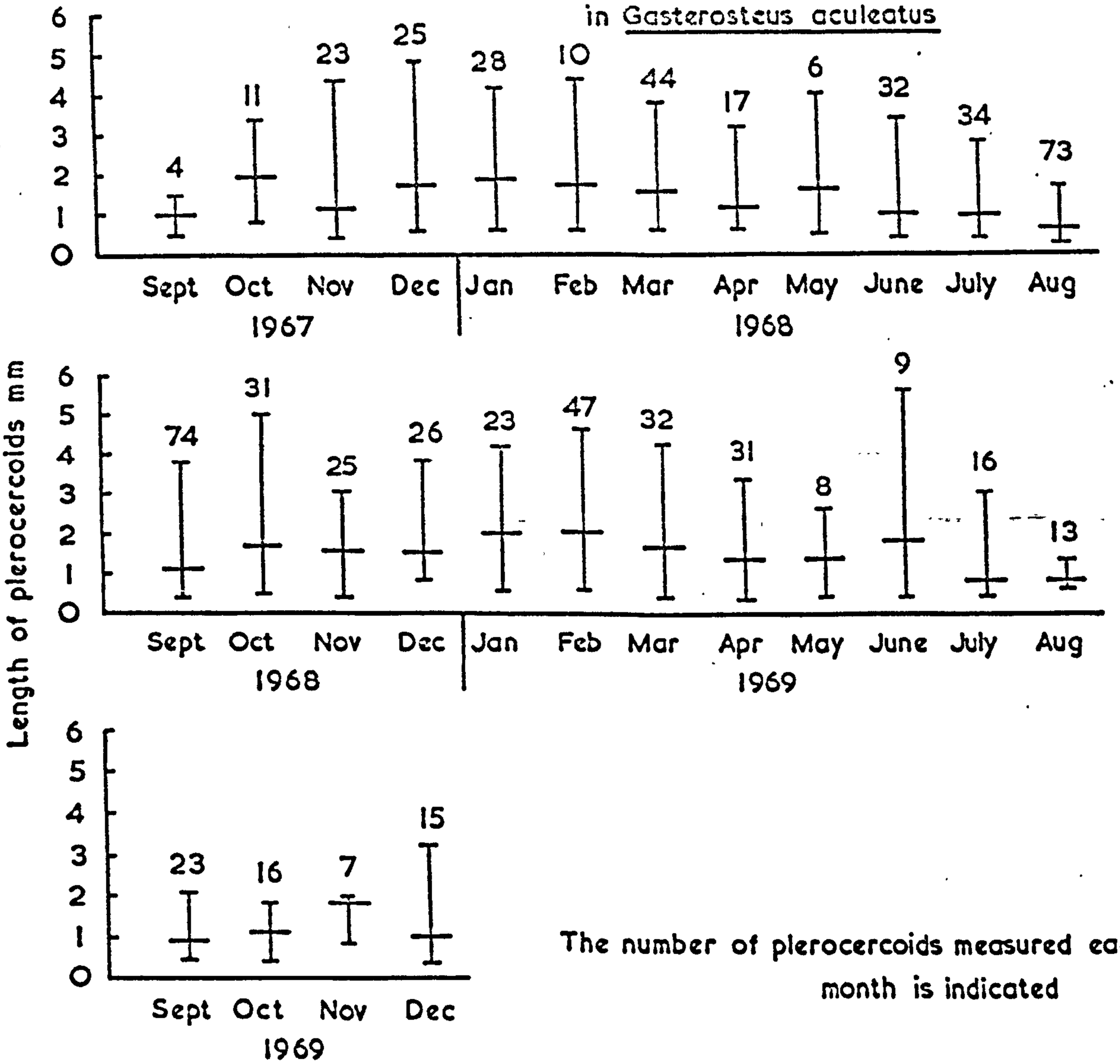
During the transitional period between plerocercoid concentration in the rectum and the intestine no correlation between length and position was observed.

6. Distribution of Proteocephalus filicollis in the fish samples

(a) Distribution according to size of fish

Fig. 11 indicates that the infection is spread among the various size groups each month. All size groups of fish harboured gravid worms. In July 1968, and July and August 1969 0^+ fish appeared in the fish samples becoming infected soon after, as very small fish. Gravid worms were even recorded to infect 0^+ fish in July 1968. Fish less than 3 cm long made up a much larger part of the samples throughout the second winter, than during the

Fig 10 The range and mean length of plerocercoids of Proteocephalus filicollis in Gasterosteus aculeatus

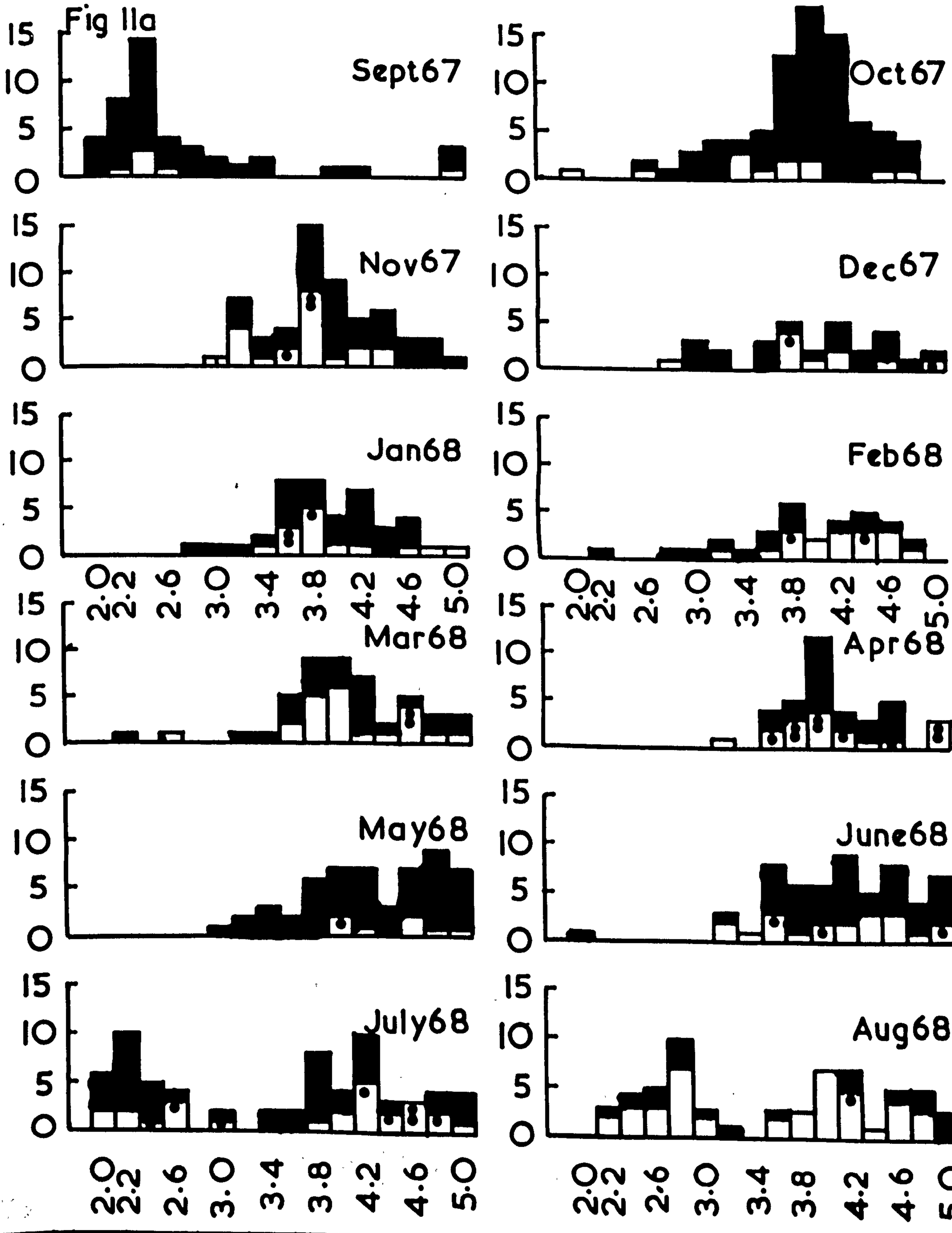


The number of plerocercoids measured each month is indicated

Figure 11

The distribution of worms in the fish population according to size of fish from October 1967 to December 1969. Black area shows the number of uninfected fish; white the number of infected fish; black dot on white area indicates a fish infected with 1 or more gravid worms with or without immature worms. The ordinate shows the number of fish, the abscissa shows the length of fish in centimetres. Each group is 2 mm., except the first column which is all fish 2 cm. and less, and the last which is all fish 5 cm. and over.

Fig 11a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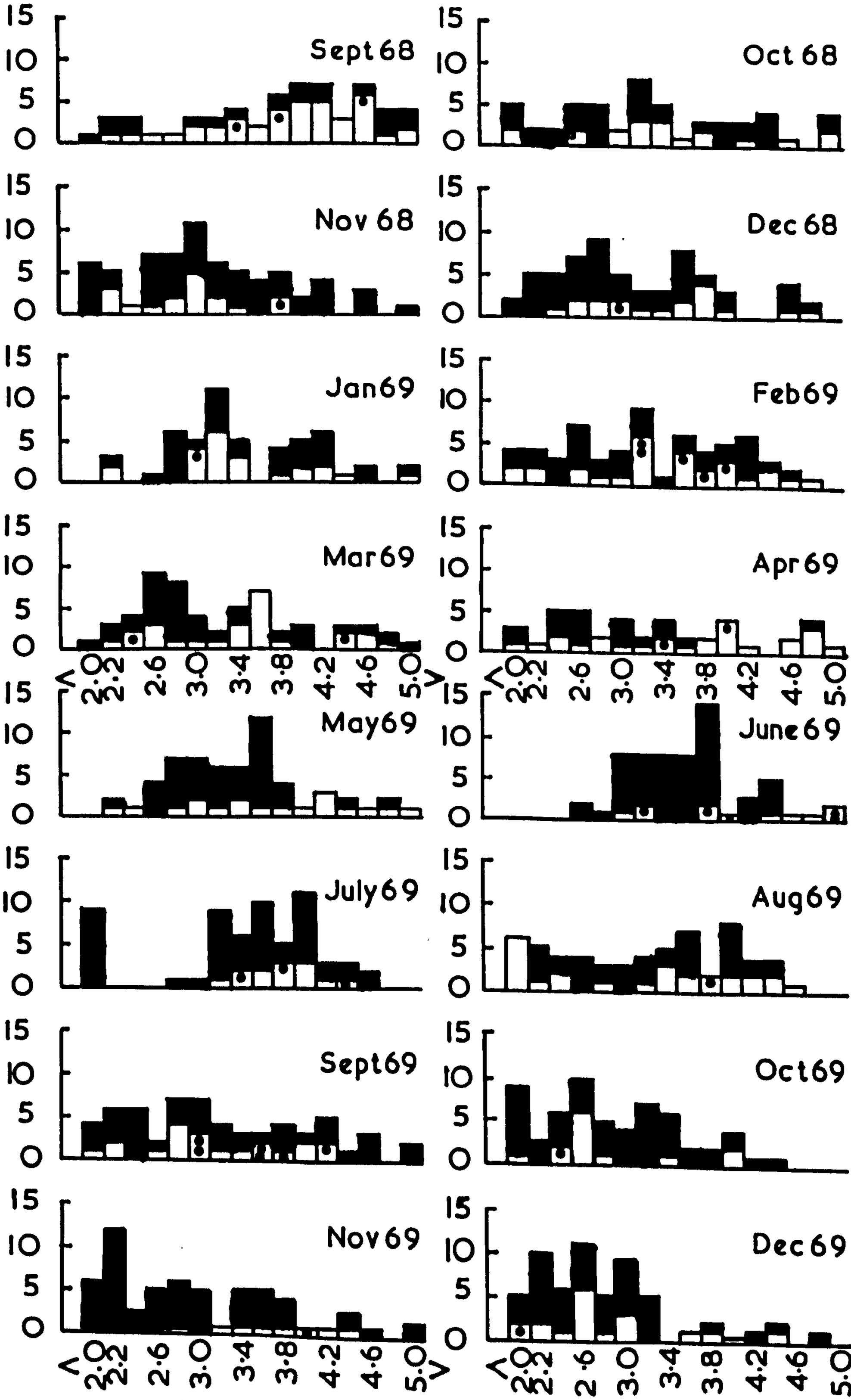
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first.

(b) Distribution according to fish sex

Of the 1,550 sticklebacks examined, 48% were female, 41% male, and 11% were not sexed. 31.5% and 30.5% of the female and male fish respectively were infected.

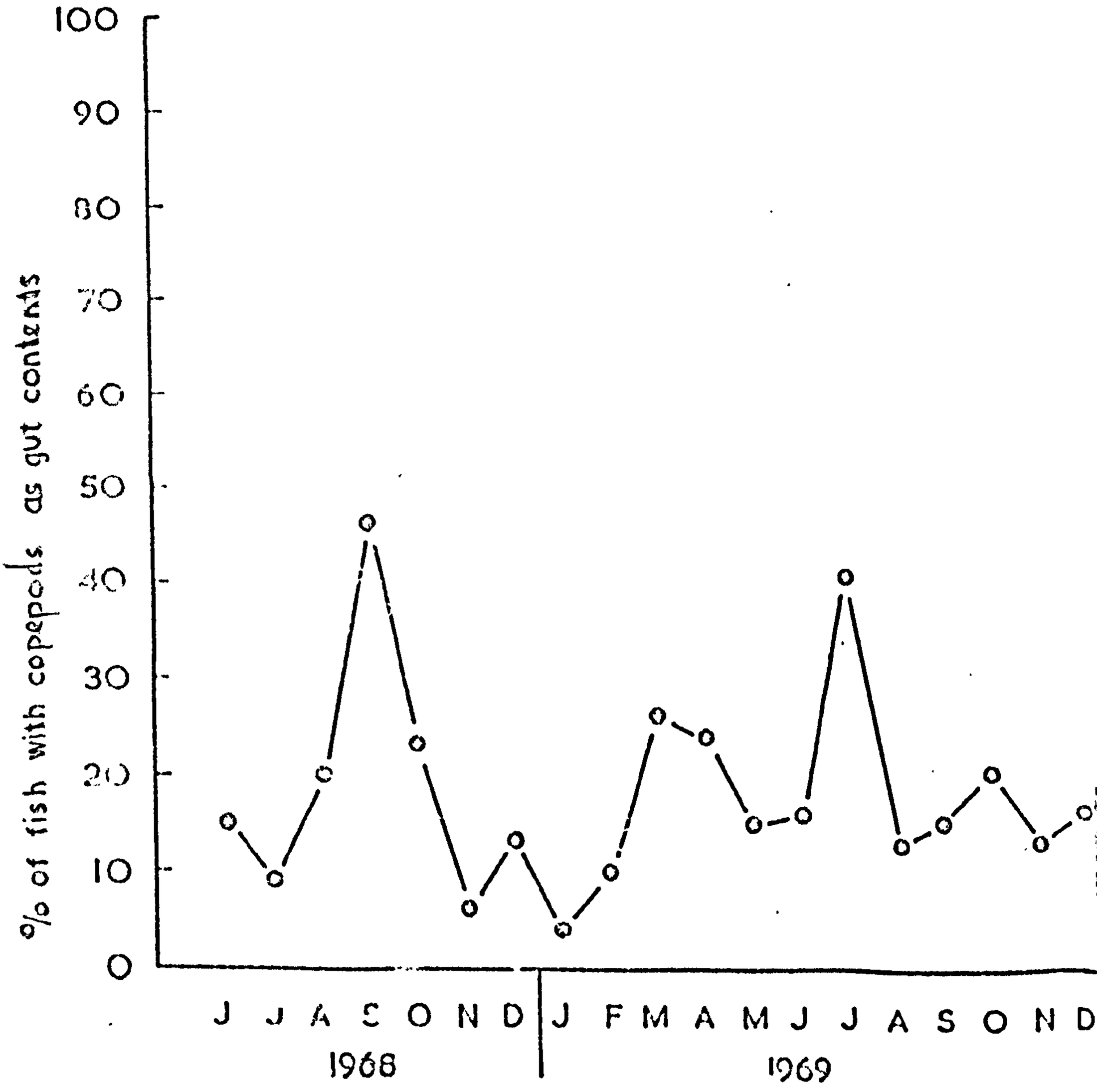
7. The diet of sticklebacks in the canal

Throughout the year the basic food of canal sticklebacks was chironomids and cladocera. Copepods made up part of the diet throughout the period June 1968 to December 1969 (Fig. 12). Over 40% of the fish had been feeding on copepods in September 1968 and July 1969. No copepod containing a proteocephalid procercoid was ever found, nor was there ever any indication of a cessation of feeding either in winter or before or during the breeding season in June and July.

8. The temperature of the canal

The water temperature of the canal was much higher in June 1968 (Table I) than in June 1969. However higher water temperatures in the region of 17°C persisted in the canal from June to September 1969 while in 1968 temperatures in the region of 15°C were recorded for the rest of the summer. Extremely low temperatures were

Fig 12 The percentage of Gasterosteus aculeatus in the canal feeding on copepods



recorded from November 1967 to March 1968, and from November 1968 to April 1969. It is apparent, however, that the canal water began to warm up in April of both years. Ice frequently covered the canal for long periods during January, February and March in both years.

DISCUSSION

(a) Maturation

Seasonal cycles of maturation have been shown for Proteocephalus torulosus in dace (Leuciscus leuciscus) in the river Avon (Kennedy and Hine, 1969), for P. stizostethi in the yellow pikeperch Stizostedion vitreum vitreum in Canada (Connor 1953), for Proteocephalus sp. in Coregonus lavaretus in Loch Lomond (Section 5), and for Proteocephalus spp. in Coregonus spp. in Lake Maggiore (Grimaldi 1964). Similarly Hopkins (1959) and Willemse (1968) have demonstrated seasonal maturation cycles for P. filicollis in Scotland and the Netherlands respectively. Thus proteocephalids of fish in temperate climates mature seasonally.

Chappell (1968), however, found that P. filicollis did not mature seasonally in a Yorkshire pond; gravid worms were present in every sample. Similarly gravid worms occurred all year round in the canal in Glasgow (Fig. 5). Thus P. filicollis in certain sites need not mature seasonally.

Hopkins (1959) found that P. filicollis became gravid in May and June, plerocercoids being acquired in July and August. These plerocercoids did not strobilate and mature until the following spring and summer. Plerocercoids

acquired by 0^+ fish in June and July in the canal became gravid that summer e.g. July 1968 or later in the year e.g. October and December 1969 (Fig. 11). The presence of gravid worms throughout most of the year (Fig. 5) also indicates that plerocercoids in the canal need not, as in the pond studied by Hopkins(1959), overwinter before strobilation and maturation.

The proximity of the canal site and the Lanarkshire pond studied by Hopkins (1959) renders any hypothesis, based on meteorological factors, explaining the different maturation patterns in the two sites, worthless. Plerocercoids in the canal were, as indicated by the rising worm burden of the fish samples (Fig. 4) and the presence of small plerocercoids (Fig. 10), acquired during the winter months. In the Lanarkshire pond (Hopkins 1959) no infections were acquired from November until July. This latter period might well, indirectly, have been partly responsible for the appearance of a seasonal maturation cycle in the Lanarkshire pond.

(b) Incidence

Hopkins (1959) found a distinct seasonal incidence cycle for P. filicollis clearly related to the seasonal maturation cycle. The absence of a seasonal incidence cycle in the canal (Fig. 2) is thus not unexpected

considering the lack of a seasonal maturation cycle. Likewise no seasonal cycle of the worm burden of the fish samples was noted (Fig. 4). The presence (Fig. 10) of small plerocercoids throughout the year suggests continuous worm recruitment. Mature and gravid worms (Fig. 5) are, however, greatly outnumbered by immature worms. Immature worms were therefore being lost at a considerable rate. Thus changes in incidence and worm burden (Figs. 2, 3 and 4) can be explained by relative changes in the rates of worm recruitment and loss. As the rate of growth of plerocercoids is unknown (Section 1) it is impossible to determine whether falling incidence is due to increased worm loss or decreased worm recruitment and vice versa. The dynamics of proteocephalid infections of fish have been discussed elsewhere (Hopkins 1959, Section 5).

Wagner (1954) showed that oncospheres of Proteocephalus tumidocollus in copepods at 4°C and below do not develop, and remain small for many weeks. When he raised the temperature to summer levels normal development of these retarded metacestodes ensued. Thus it is likely that P. filicollis eggs released in January and February (Fig. 5) and ingested by copepods would only become fully infective procercoids in summer. The continuous

plerocercoid recruitment and the continuous release of eggs suggest that, in the canal at least, there exists an ever present pool of procercoids in the copepod population. Evidence for the presence of procercoids of a species of Proteocephalus infecting Coregonus lavaretus in the copepod population for most of the year was presented in Section 5 . Future investigations into the dynamics of fish tapeworms should clearly involve study of the worm infection in both the fish and copepod hosts.

(c) Worm location in the stickleback intestine

1. Plerocercoids

In the Lanarkshire pond studied by Hopkins (1959) plerocercoids, which occurred only from July to March, were virtually all attached in the rectum. Similarly Willemse (1968) stated that all plerocercoids were located in the rectum. Only 2 plerocercoids, however, were found by Chappell (1969) in the rectum. P. filicollis plerocercoids in the canal showed a seasonal distribution pattern down the length of the fish gut (Fig. 7). It was shown in Section 1 that, at 15°C, procercoids initially attach as plerocercoids in the anterior and mid-intestine regions. Likewise it is clear that plerocercoids attach initially anterior to the ileo-rectal

valve from February to July 1968, and from March to August 1969. However, in September and October 1967, from August to December 1968 and from September to December 1969 it would seem that initial attachment was occurring in the rectum. The ability of plerocercoids to migrate has been suggested by Hopkins (1959) for P. filicollis, shown in Section 5 for Proteocephalus sp. in Coregonus lavaretus, and shown experimentally by Wagner (1954) for P. tumidocollus in trout. Thus it would seem that the initial position of attachment has little bearing on the position which a plerocercoid takes up to complete its development.

2. Strobilate worms

There was, however, no apparent spatial seasonal distribution of strobilate worms within the stickleback gut. Since 3 of the 4 strobilate worms (Fig. 6) attached in the rectum, were gravid, it seems probable, especially since their strobilae were outwith the intestine in the water, that these worms were in the process of being lost.

Extreme anterior attachment of gravid P. filicollis in the stickleback gut was noted principally by Hopkins (1959). The great majority of worms attached close to the pyloric valve in this study were gravid. The adults

of other proteocephalids (Gresson & Corbett 1954, Willemse 1968 and Section 5) apparently also attach anteriorly. Anterior attachment presumably provides a greater length of suitable intestine for strobila development. In this connection it is interesting that Bråten & Hopkins (1969) have found that, as Hymenolepis diminuta grows, it attaches more anteriorly within the rat intestine thus maintaining its strobila in a physiologically favorable intestinal region.

The strobilae of large gravid P. filicollis are in general longer than the 4 anterior intestinal regions combined, especially in smaller fish, resulting in the formation of a U-bend in the strobila near the ileo-rectal valve, the posterior segments being directed anteriorly instead of posteriorly in the intestine. Strobilate worms were never found, irrespective of their position of attachment in the 4 anterior intestine regions, with their posterior segments lying in the rectum. This either means that the rectum is physiologically unfavourable to proglottids of strobilate worms, or passive passage of tapeworm strobilae through the ileo-caecal valve is impossible. Meggitt (1914) found, as did the present author, the occasional occurrence of large gravid P.

filicollis shedding their eggs into the water with their scoleces still attached within the fish rectum. He believed that, once ready to release its eggs, a gravid worm migrated down the intestine and became attached posteriorly letting its strobila hang freely in the water. Having released most of its eggs the whole worm was then lost. Meggitt thought that the posterior migration was passive being due to peristaltic movements of the fish gut. It now seems more likely, however, that, since gravid worms were attached in all gut regions there is migration of the scoleces of gravid worms from the anterior regions to the rectum. This view is supported by the evidence and also by the more general realisation (Bråten & Hopkins 1969, Hopkins 1970) that tapeworms are by no means parasites incapable of independant specifically orientated movement within the gut environment.

Since strobilate worms were virtually confined to the 4 anterior intestine regions while plerocercoids occurred both in the rectum and the 4 anterior regions it seems reasonable to state that strobilation can only occur in the latter regions. It is impossible to say, however, whether the anterior regions stimulate strobilation or whether the rectal environment inhibits it. It has been suggested in Section 5 that plerocercoids of

Proteocephalus sp. in Coregonus lavaretus do not strobilate until their scoleces are attached within the pyloric caecae.

(d) The onset of strobilation

Hopkins (1959) stated that most plerocercoids strobilate when 5-6 mm, while Chappell (1969) found that strobilation first appeared in plerocercoids 1.3 - 2.9 mm. The smallest strobilate worm recorded in this study was 2.08 mm and possessed 4 visible segments. One cannot, however, from this evidence state that P. filicollis in the canal strobilates when 2.08 mm. Many plerocercoids over twice as long were found showing no indication of strobilation. Throughout the two year period 13 worms with 5 segments or less and probably newly strobilate were measured. They ranged from 2.08 to 5.5 mm, mean 3.4 mm. Most worms less than 3.4 mm were unsegmented plerocercoids, while, although the great majority of worms greater than 3.4 mm were strobilate, a considerable number were still plerocercoids. Thus one can conclude that strobilation in canal sticklebacks at least is unlikely to occur in worms much smaller than 3.4 mm, but need not occur until plerocercoids are considerably longer. Thus plerocercoid size is considered

not to be wholy responsible for the onset of strobilation, other factors such as worm position and host physiological condition being probably partly responsible. That position is important was shown earlier by the fact that plerocercoids never strobilate in the rectum. Plerocercoids were recorded as being attached in the rectum from July until the following spring by Hopkins (1959). This long stay in the rectum might well explain why his plerocercoids were so large (5 to 6 mm) when strobilation occurred in the intestine in spring.

(e) <u>The relationship of worm length, segment number and maturation</u>

As the length and segment number of worms increased, so also did their maturity (Fig. 8 & 9). Since a large overlap of worm length and proglottid counts of gravid and non-gravid worms occurred it is clear that egg production is a direct function of neither worm size nor segment number. Worms with less than 13 segments or shorter than 6 mm were, however, unlikely to be gravid.

(f) <u>Worm distribution in the fish population</u>

Considering the similar incidence of <u>Proteocephalus filicollis</u> in both sexes and the relatively even spread of the infection throughout the various size groups of fish

each month (Fig. 11) it is reasonable to conclude that both sexes and all sizes (and ages) of sticklebacks in the canal are equally liable to proteocephalid infection.

SUMMARY

(1) The incidence, intensity of infection and development of Proteocephalus filicollis was studied by the monthly examination of approximately 60 Gasterosteus aculeatus in a Glasgow canal over a 27 month period.

(2) No seasonal maturation nor incidence cycle was apparent.

(3) Plerocercoids showed a seasonal distribution pattern down the length of the stickleback intestine. Plerocercoids were concentrated in the rectum in September and October 1967, from August to December 1968, and from September to December 1969. From February to July 1968, and from March to August 1969 plerocercoids were concentrated anterior to the ileo-rectal valve.

(4) Strobilate worms showed no seasonal distribution pattern down the intestine. Gravid worms were concentrated in the most anterior intestinal regions.

(5) Gravid worms were generally longer and possessed more segments than non-gravid worms. Although the onset of strobilation is not apparently directly dependant on plerocercoid size, it is calculated that plerocercoids in

the canal are most likely to strobilate when 3.4 mm.

(6) The Proteocephalus filicollis infection was spread evenly throughout the fish samples.

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SECTION 3

Investigations into the biology of Proteocephalus filicollis (Rud.1810) a cestode parasite of the three-spined stickleback, Gasterosteus aculeatus (L.)

(3) Incidence and maturation in a pond in Glasgow.

(with 11 figures in the text)

INDEX

INTRODUCTION

This study of the life cycle of Proteocephalus filicollis in three-spined sticklebacks, Gasterosteus aculeatus in a Glasgow pond, forms the second part of an investigation into the ecology of the cestode in the Glasgow area. The first section of the work (see Section 2) was executed simultaneously.

A distinct seasonal cycle of incidence and maturation has been reported for P. filicollis by Hopkins (1959) and Willemse (1968). In the canal (see Section 2) no maturation cycle occurred, gravid worms being present in most samples.

MATERIALS AND METHODS

1. The pond

Proteocephalus filicollis occurred as a natural infection of three-spined sticklebacks, *Gasterosteus aculeatus* in an artificial pond in a public park at Springburn in the north of Glasgow. The pond lies 250 ft. above sea level, measures approximately 150 metres long by 40 metres wide, and is very shallow, never more than 1 metre deep. The pond was extremely eutrophic being well weeded, with the filamentous alga *Spirogyra* abundant throughout the year in protected parts of the pond. Many types of invertebrates were noted, while the sole vertebrate in the pond was the three-spined stickleback.

2. Collection and examination of fish

From October 1967 to September 1969 approximately 60 fish were caught each month using a 4 ft. beam trawl. The pond temperature at a depth of 15 cm was noted as each sample was taken. The fish were taken to the laboratory, kept in running water and examined, their diet noted, and the worms' position, condition, and length recorded as in Sections 1.2. A qualitative inspection of the gut contents was made during the latter half of the survey. Difficulty in catching sufficient fish was experienced in December 1967

when only 19 were caught, and a 15 cm thick ice cover in February 1968 prevented any fish being caught.

RESULTS

Details of the numbers of fish caught, the numbers infected, the numbers of worms found, the incidence and mean worm burden each month are presented, along with the monthly temperature records in Table I.

1. Incidence and mean worm burden

The incidence of Proteocephalus filicollis in pond sticklebacks (Fig. 1) was low from October 1967 to March 1968, increased to 23% in April 1968, and then fell in May and June 1968. The incidence soared to 68% in July 1968 before falling to relatively low levels in the following two months. From October 1968 to April 1969 the incidence remained high, always over 35%, falling eventually in May and June 1969. From the low June level the incidence increased during summer and reached 30% by September 1969.

As shown in Fig. 2 the mean worm burden (i.e. the number of worms per infected fish) remained low from October 1967 to March 1968, rose to 2.5 in April 1968 and reached a peak of 7 in July 1968. The mean worm burden

Table I Incidence and mean worm burden of Proteocephalus filicollis in the three spined stickleback (G.aculeatus) in a duck pond at Springburn

Month and year	No.of sticklebacks examd.	Infected	Infected %	No.of worms found	Mean worm burden /Infected fish	Temperature °C
1967						
Oct	179	26	15	40	1·5	7.0
Nov	67	4	6	6	1·5	40
Dec	17	0	0	0	0·0	4.0
1968						
Jan	42	2	5	2	1·0	50
Feb	No sample					
Mar	50	4	8	4	1·0	5.5
Apr	40	9	23	23	2·5	9.0
May	46	7	15	14	20	10·0
June	70	6	9	11	18	13·0
July	60	41	68	289	7·0	17.0
Aug	81	20	25	34	1·7	20.0
Sept	67	13	19	21	1·6	16.0
Oct	68	29	43	62	2·1	12·0
Nov	55	29	53	60	2.0	4.0
Dec	55	19	35	40	2·1	6.0
1969						
Jan	58	30	52	79	2·6	2·5
Feb	70	31	44	70	2·2	2·5
Mar	58	21	36	37	1·7	3·0
Apr	58	22	38	56	2·5	5·0
May	59	10	17	39	3·9	7·5
June	60	4	7·0	7	1·7	12·0
July	60	12	20	19	1·5	10·5
Aug	56	12	21	15	1·2	19·0
Sept	59	17	29	22	1·2	15·0

Fig 1 The incidence of infection of Proteocephalus filicollis in Gasterosteus aculeatus each month from October 1967 to September 1969

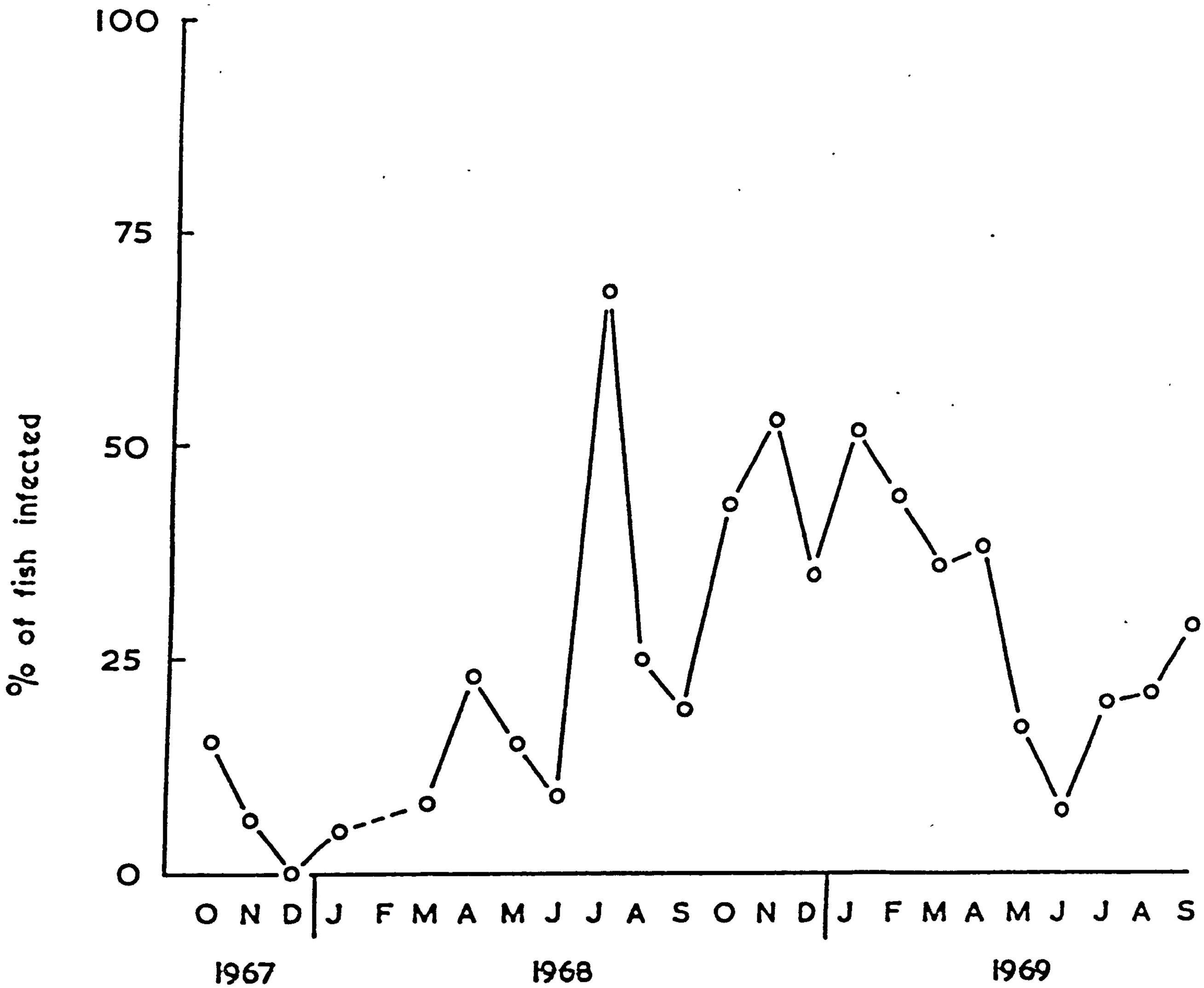
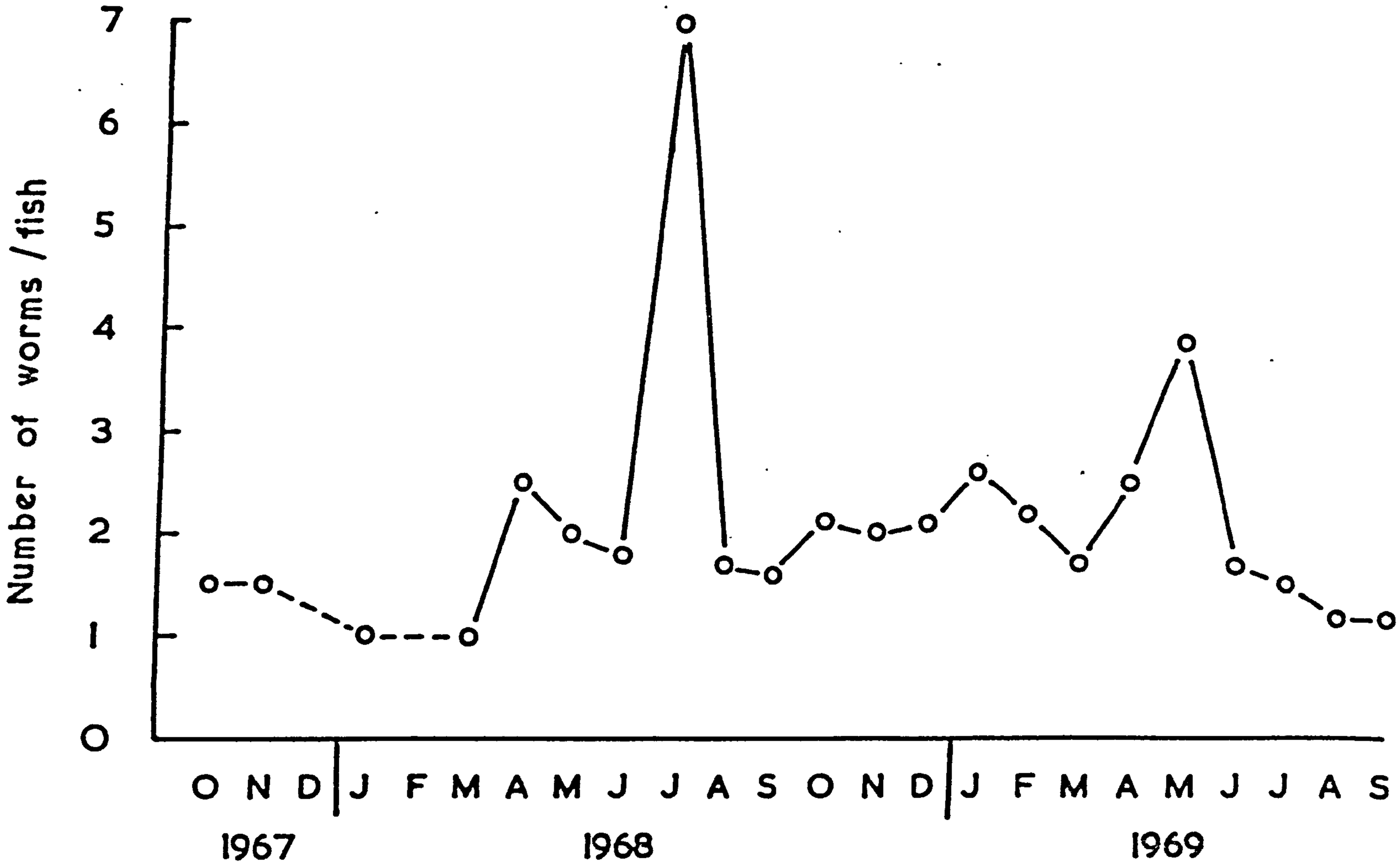


Fig 2 The mean worm burden of Proteocephalus filicollis in Gasterosteus aculeatus each month from October 1967 to September 1969



fell to 1.7 in August 1968. From September 1968 to April 1969 the mean worm burden remained over 2, rose to 3.9 in May 1969, and then fell to remain below 2 throughout summer 1969.

As in the canal survey (Section 2) the incidence and the mean worm burden did not always rise and fall in unison, a phenomenon discussed in Section 2 . A study of the product of the incidence and mean worm burden (the worm burden of the fish sample) yields, however, a clearer picture of the dynamics of the worm population.

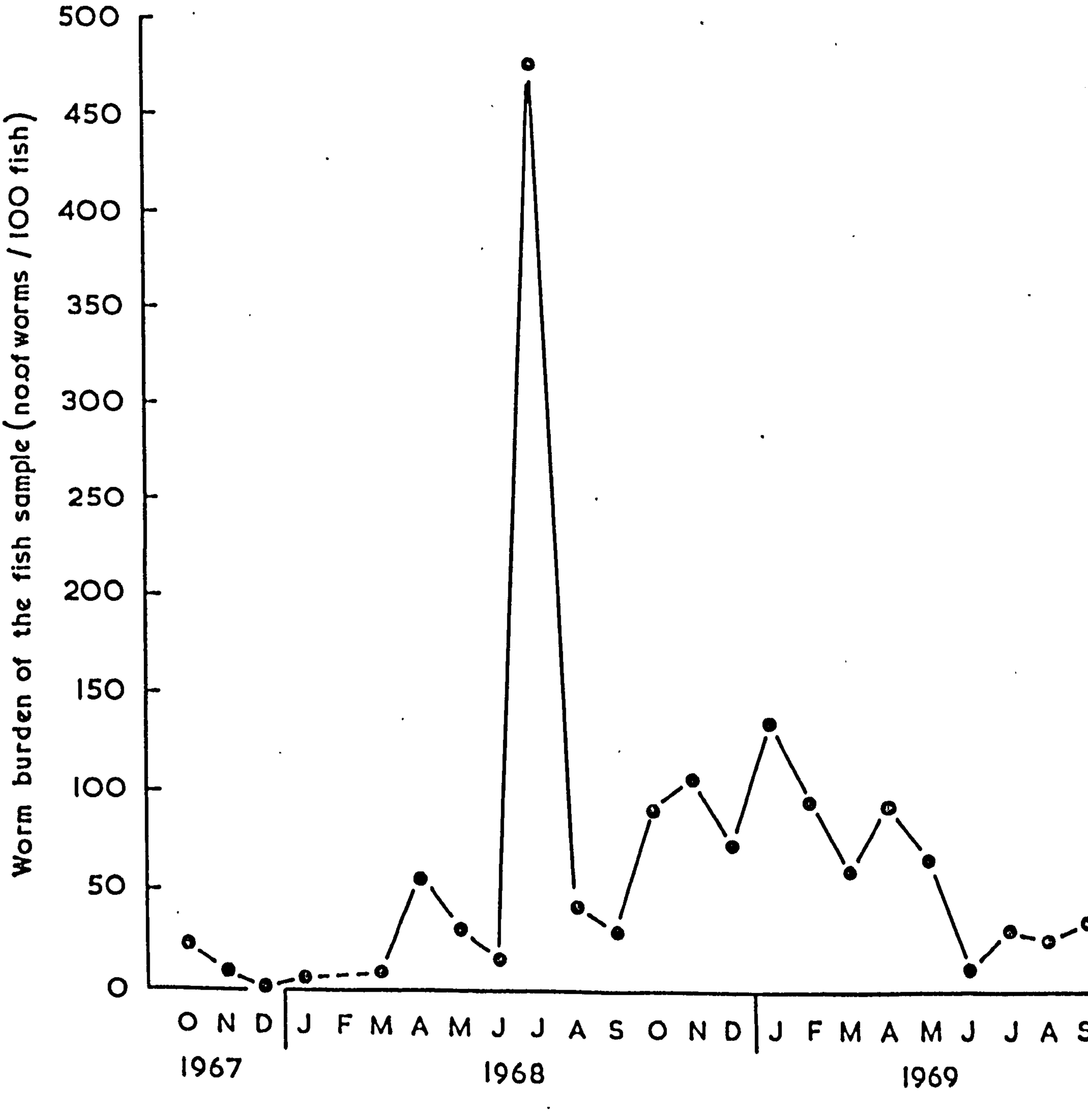
2. The worm burden of the fish sample

The tremendous increase in the worm burden of the fish sample in July 1968, its collapse in August 1968, and its failure to repeat itself in the following year are the most outstanding features of Fig. 3. The worm burden of the fish sample was low during the first autumn and winter compared to the relatively high levels which occurred over the corresponding period of the second year.

3. Worm maturation

Plerocercoids were relatively rare in the worm

Fig 3 The Proteocephalus filicollis worm burden in Gasterosteus aculeatus each month from October 1967 to September 1969



sample in November 1967, May and September 1968 and totally absent in August 1969 (Fig.4) Strobilate non-gravid worms occurred each month except January and March 1968 when few worms at all occurred. From April to August 1968 and in June 1969 strobilate non-gravid worms contributed greatly to the strobilate worm population. Gravid worms were common in October 1967, and prominent in November 1967, and January and March 1968 when the worm burden of the fish sample (Fig.3) was very low. Although rare from April to August 1968, gravid worms were common from September 1968 to September 1969,although absent in June 1969. The gravid worm population, however, recovered in late summer.

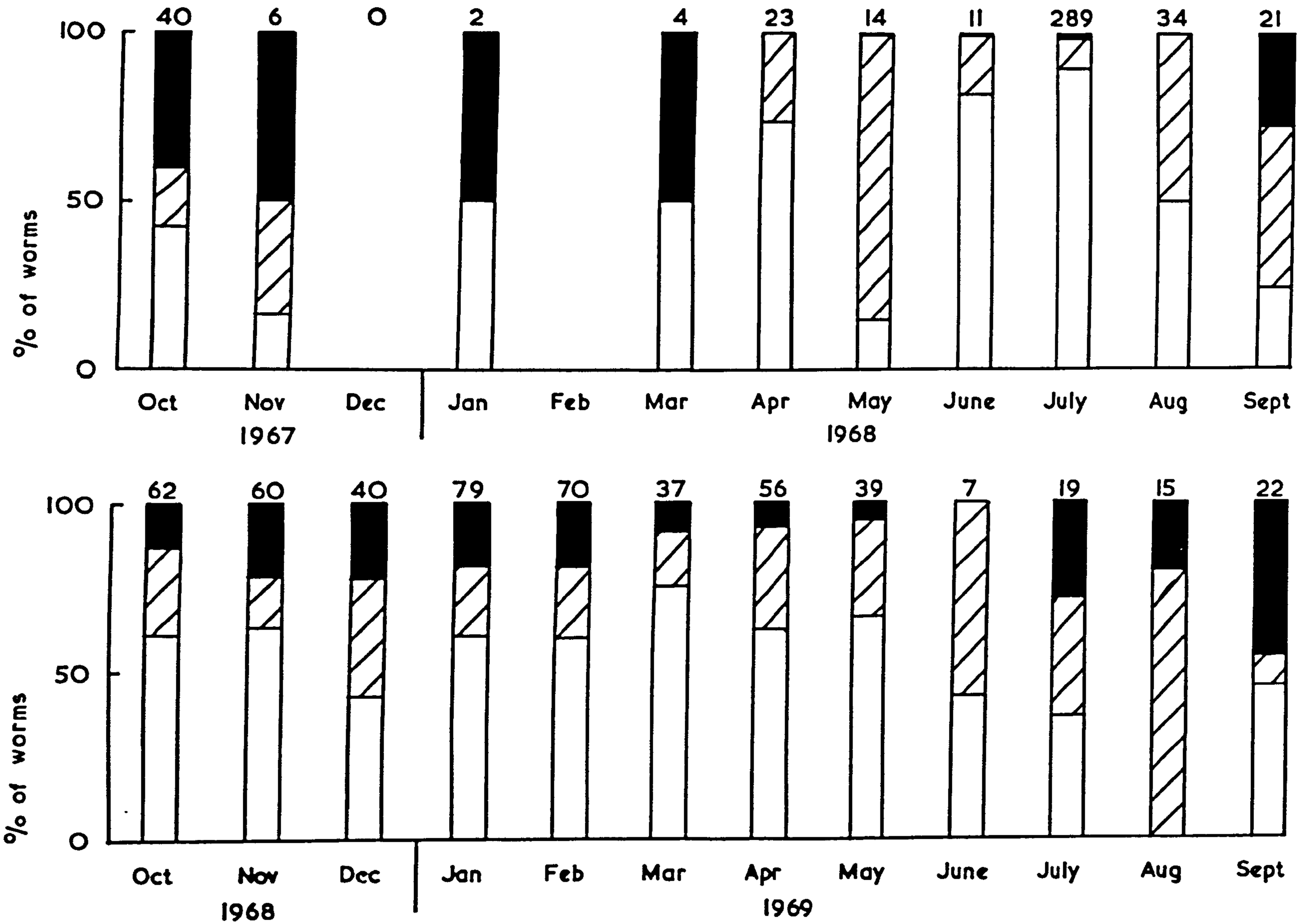
4. Worm position

(a) Strobilate worms

Strobilate worms occurred in all regions of the stickleback gut (Fig.5), being most prevalent in the anterior intestine where 43.2% occurred, while 33.2% were attached in the mid-intestine. Very few strobilate worms (0.6% or 2 of the 324 strobilate worms recorded) were found in the rectum.

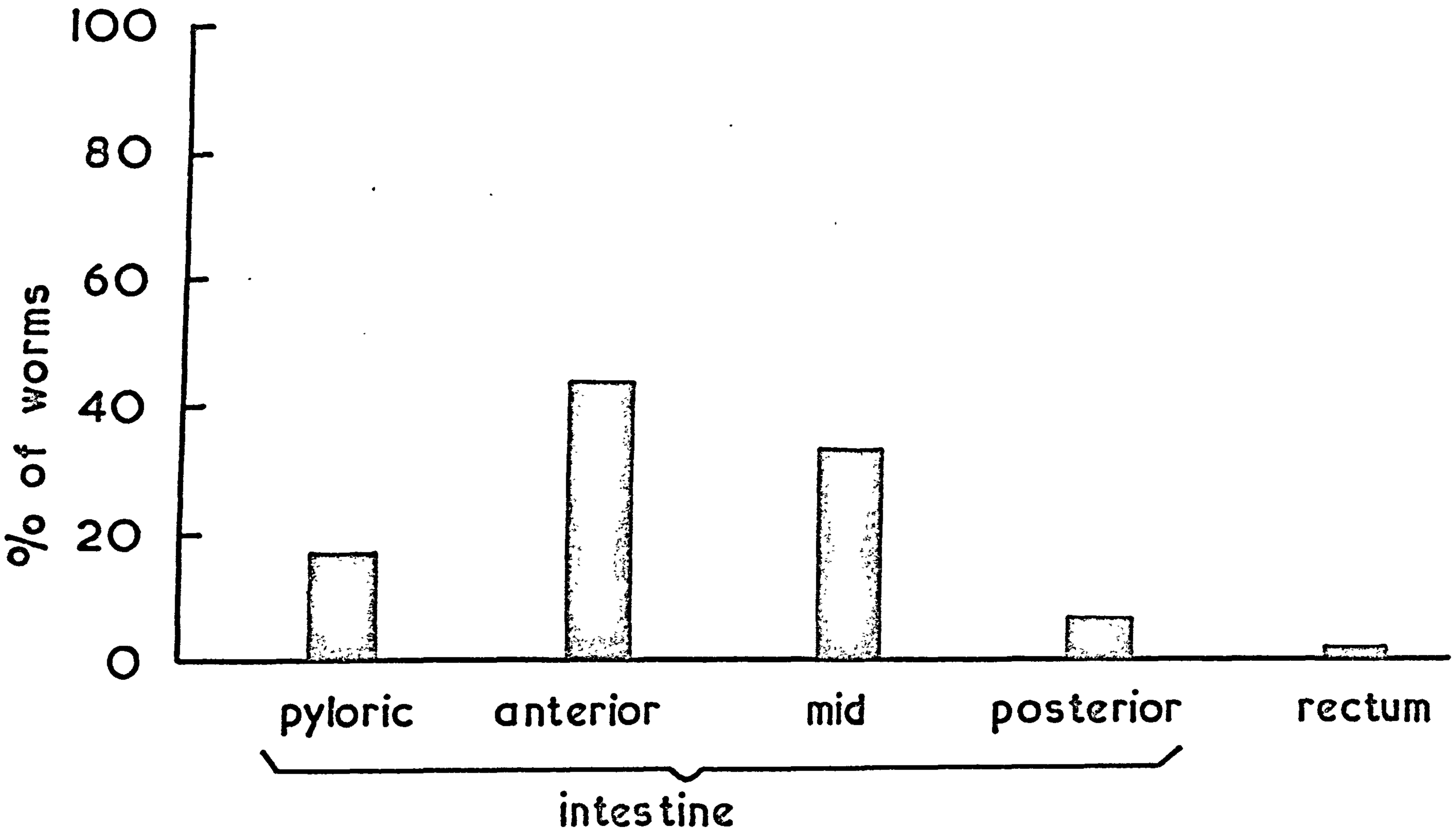
Gravid, mature and immature worms occurred in all regions. Over 70% of the gravid worms occurred in the

Fig 4 The percentage of plerocercoids (clear), strobilate non-gravid (crosshatched) and gravid (black) *Proteocephalus fillicollis* in *Gasterosteus aculeatus* from October 1967 to September 1969



The total no.of worms found each month is shown above each column

Fig 5 The percentage of strobilate Proteocephalus filicollis attached in each gut region of Gasterosteus aculeatus



two anterior regions with 72.7% of all worms found in the pyloric region being gravid. Mature and immature worms were evenly dispersed between the anterior and mid-intestine regions, while 60% of all worms in the posterior intestine were gravid. No seasonal distribution of the strobilate worm population down the length of the stickleback gut occurred.

(b) Plerocercoids

Nearly 70% of the 627 plerocercoids recorded during the survey were attached in the mid-intestine(Fig. 6) while only 5.9% and 0.6% were attached in the rectum and pyloric region respectively. The scarcity of plerocercoids in the rectum, the apparent lack of a seasonal distribution of plerocercoids between the rectum and the anterior gut regions rendered the study of plerocercoid position in relation to season superfluous.

5. Worm size, segmentation and maturity

(a) Strobilate worms

As indicated in Fig.7 gravid worms were generally longer than non-gravid worms. Gravid and non-gravid worms ranged in length between 6.4 mm and 35.2 mm, and 1 mm and 15.8 mm respectively. Thus between 6.4 mm and 15.8 mm, worms were either non-gravid or gravid.

Fig 6 The percentage of plerocercoid Proteocephalus filicollis attached in each gut region of Gasterosteus aculeatus

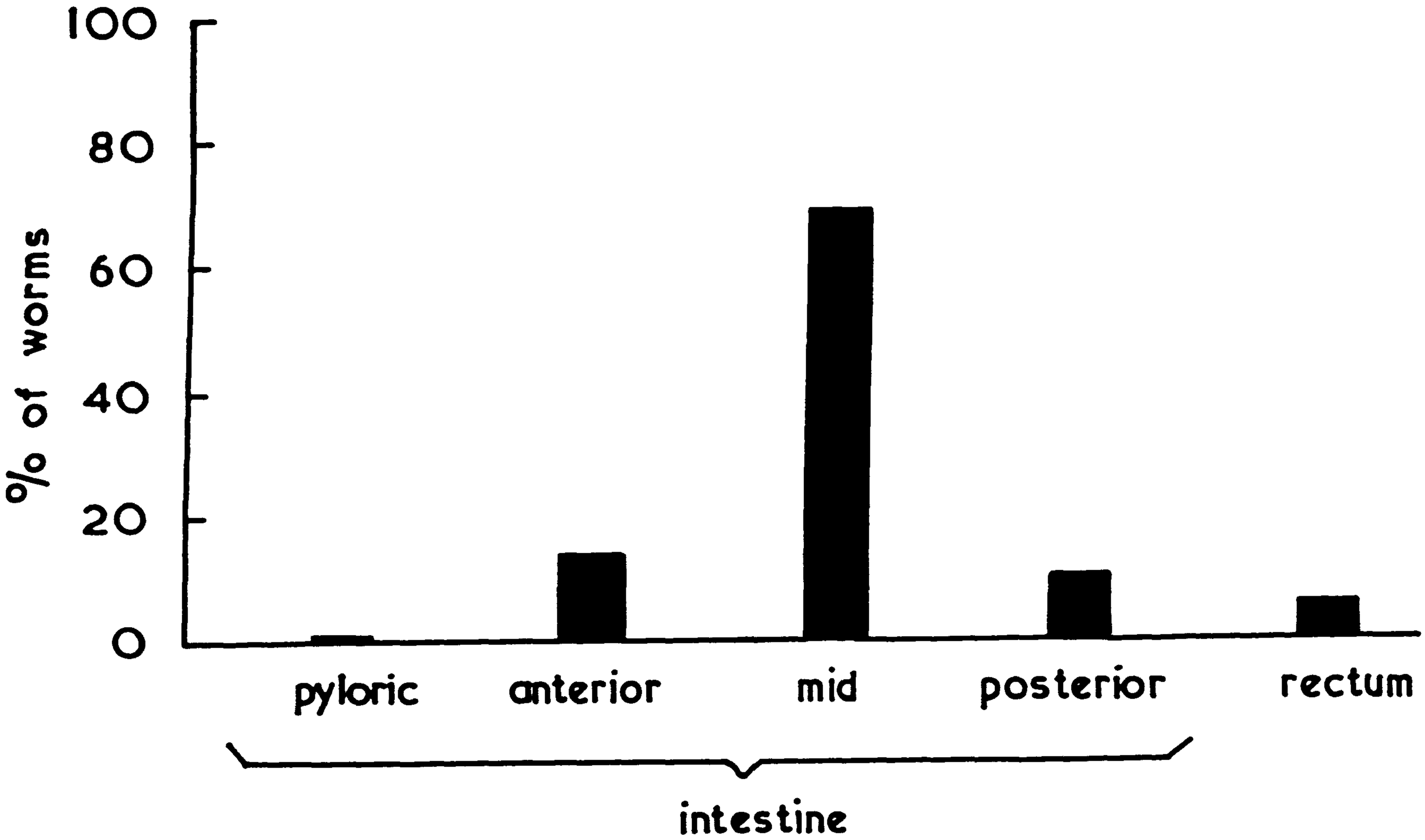
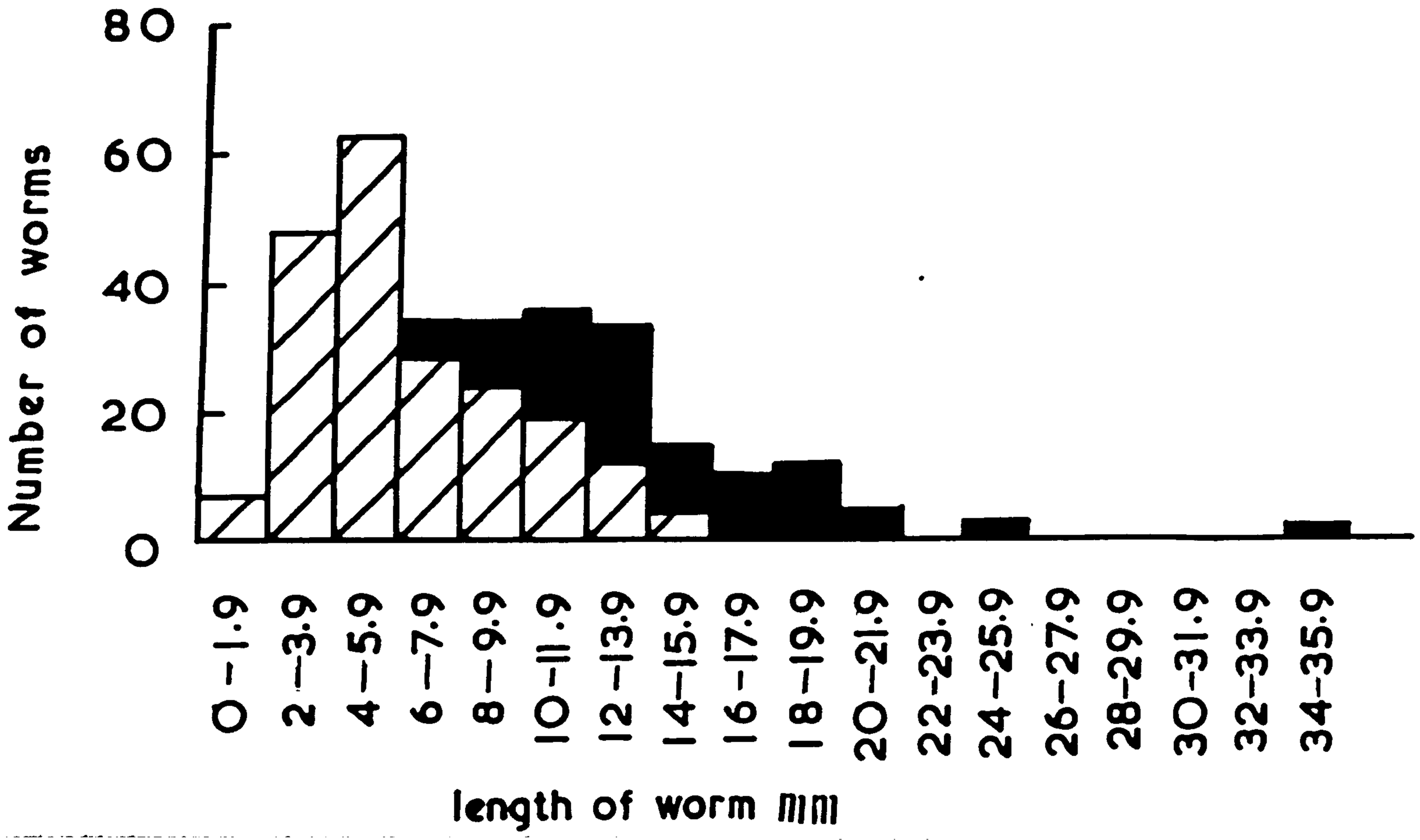


Fig 7 The relationship between length and maturity of strobilate Proteocephalus fillicollis. The crosshatched area shows the number of non-gravid worms. The black area shows the the number of gravid worms



Gravid and non-gravid worms possessed between 12 and 44, and 2 and 24 segments respectively (Fig. 8). Worms with between 12 and 24 segments were either gravid or non-gravid the worms with more segments being more likely to be gravid.

(b) Plerocercoid length

Plerocercoids ranged in length from 0.32 mm to 4.9 mm, mean 1.28 mm (537 measured). Although only a few plerocercoids occurred in many months (Fig. 9) individuals less than 1 mm long were present in most samples. Large plerocercoids, over 2 mm, were relatively frequent in October 1967, April 1968, and January and February 1969.

6. The distribution of P. filicollis in the fish samples

(a) Distribution according to size of fish

The worm infections were evenly spread throughout the various size groups of fish caught most months (Fig. 10). Although gravid worms occurred basically in the smaller fish during the autumn and winter of the second year when such fish made up the bulk of the catch it seems likely considering other months, e.g. October 1967, that gravid worms had an equal chance of occurring in small and large fish.

Fig8 The relationship between the number of segments of Proteocephalus filicollis and maturity. The crosshatched area shows the number of non-gravid worms. The black area shows the number of gravid worms.

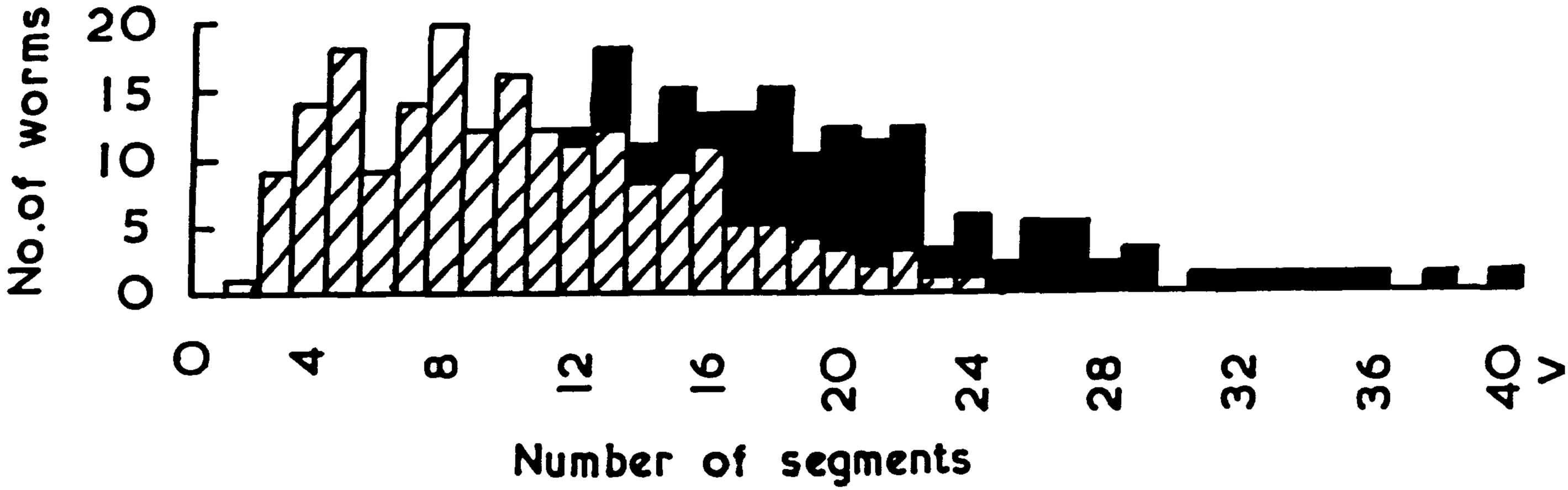
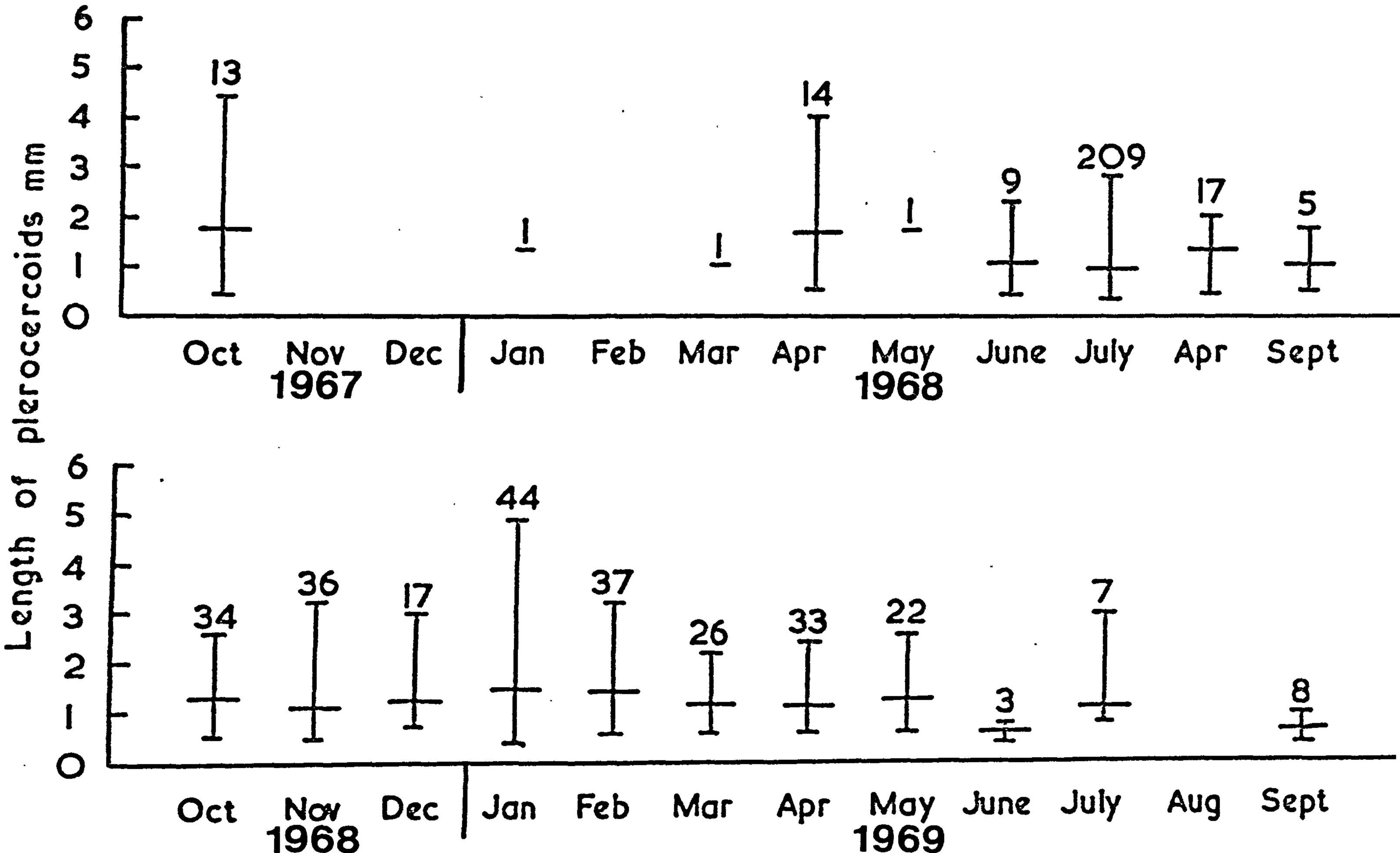


Fig 9 The mean length and length range of plerocercoids of Proteocephalus filicolli in Gasterosteus aculeatus each month from October 1967 to September 1969



The number of plerocercoids measured each month is indicated

In July of each year 0+ fish first appeared in the fish samples becoming infected as very small fish.

(b) Distribution according to fish sex

Of the 1,421 sticklebacks examined, 693 were female, 369 male, and 359 undetermined. The large number of unsexed fish is explained by the difficulty in sexing the high numbers of very small fish caught in some months (Fig. 10). 20.7% and 26.5% of the females and males respectively were infected.

7. The diet of sticklebacks in the pond

Chironomid larvae and various species of cladocera made up the bulk of the stickleback diet during the survey. Over 20% of the fish in June and December 1968, and from February to May 1969 had been eating copepods (Fig. 11). The peak level of copepod ingestion was March 1969. Few fish were feeding on copepods in August and September 1968, and July and August 1969. No copepod containing a proteocephalid procercoid was ever noted, nor was there ever any indication of a cessation of feeding either in winter, or before, or during the breeding season in summer.

8. The temperature of the pond

From November to March each year very low temperatures prevailed (Table I). The pond began to warm up in April

Figure 10

The distribution of worms in the fish population according to size of fish from October 1967 to September 1969. Black area shows the number of uninfected fish; white area the number of infected fish; black dot on white area indicates a fish infected with one or more gravid worms with or without immature worms. The ordinate shows the number of fish, the abscissa shows the length of fish in centimetres. Each group is 2 mm, except the first column which is all fish 2 cm and less, and the last which is all fish 5 cm and over.

Fig10a

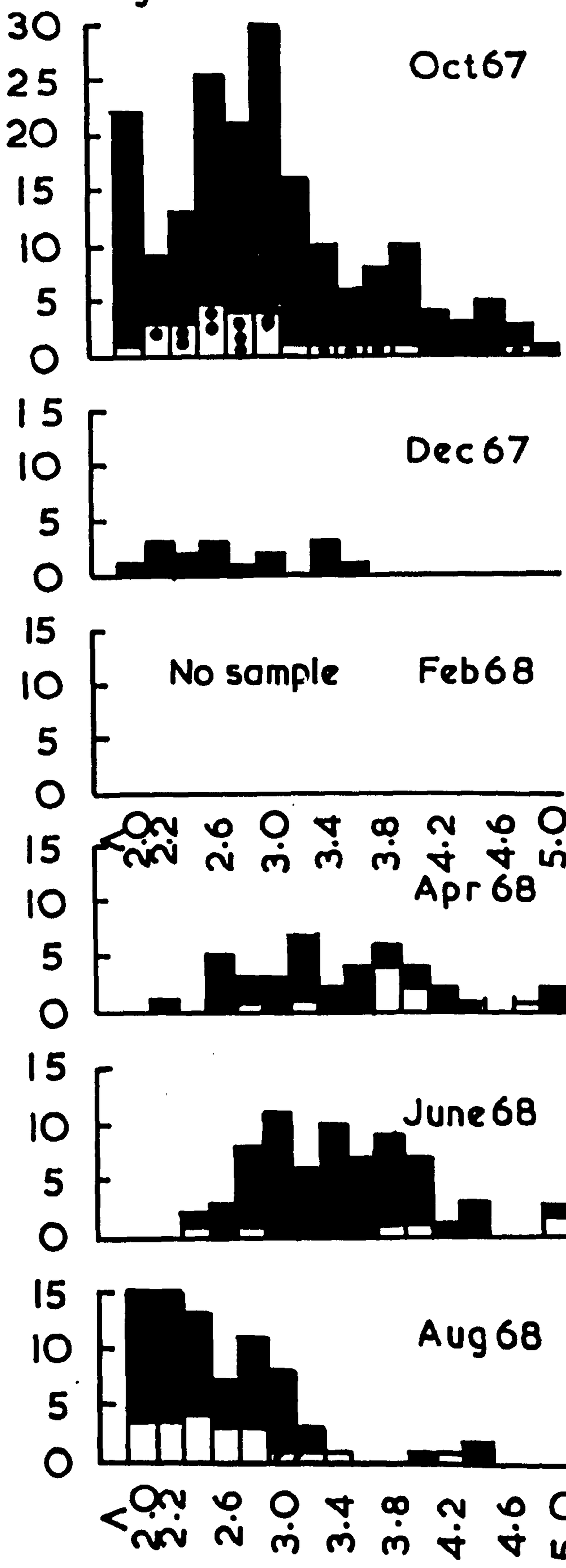

Oct67
Nov 67
Dec 67
Jan 68
No sample
Feb 68
Mar 68
Apr 68
May 68
June 68
July 68
Aug 68
Sept 68
<2.0
2.2
2.6
3.0
3.4
3.8
4.2
4.6
5.0>
0
5
10
15
20
25
30

Fig10 b

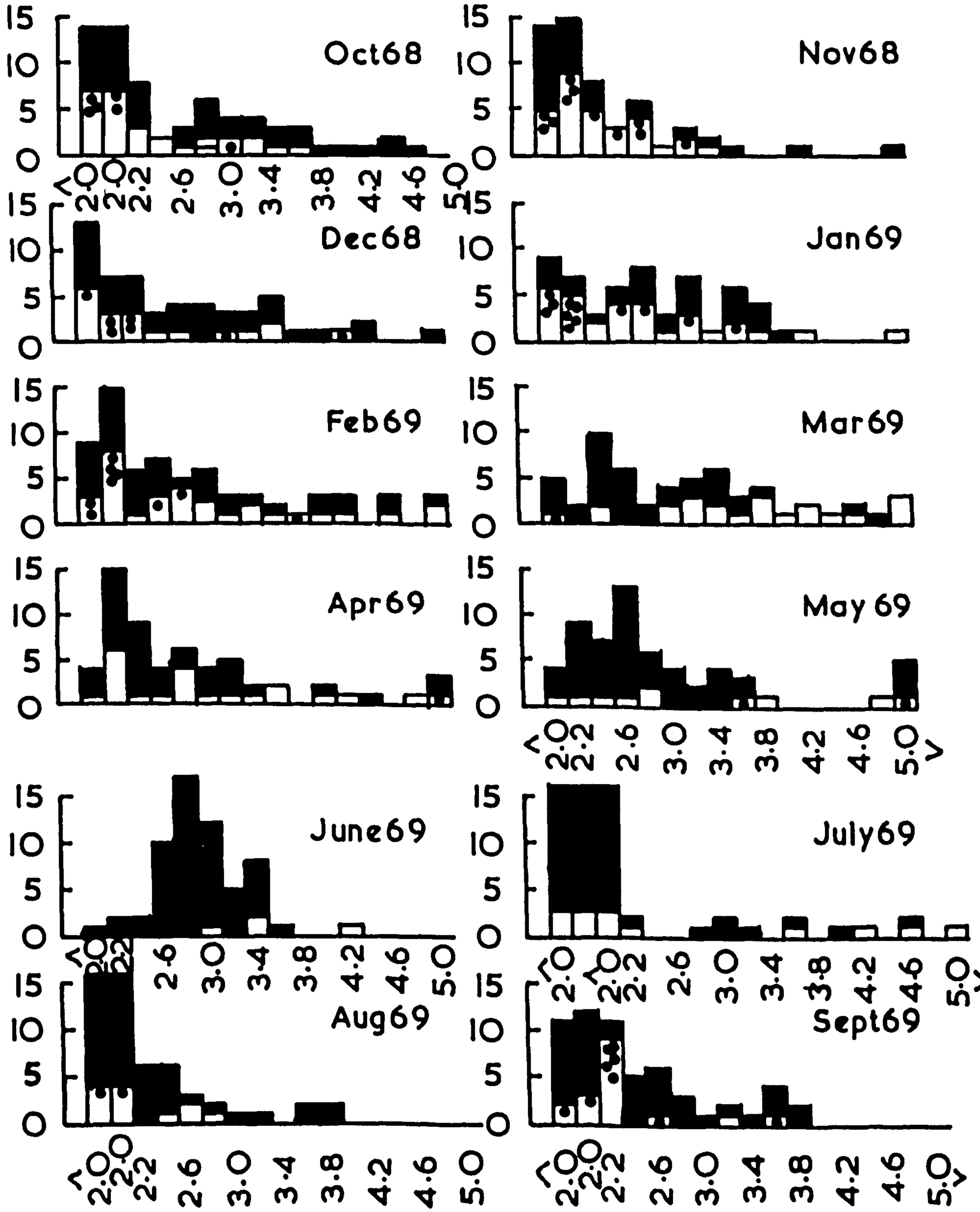
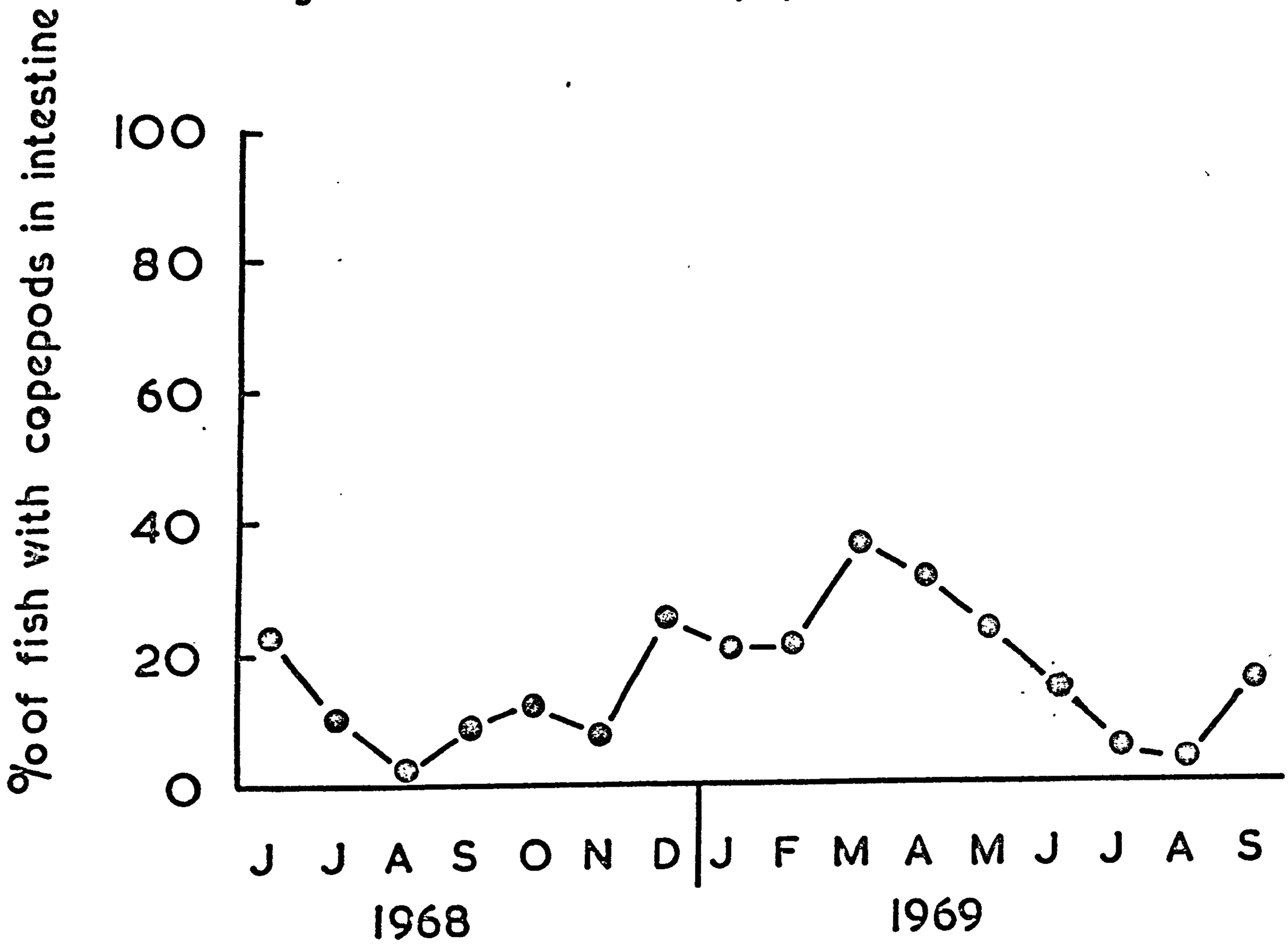

Oct68
Nov68
Dec68
Jan69
Feb69
Mar69
Apr69
May 69
June69
July69
Aug69
Sept69
15
10
5
0
<2.0
2.2
2.6
3.0
3.4
3.8
4.2
4.6
5.0
>5.0

Fig 11 The percentage of Gasterosteus aculeatus in the pond feeding each month on copepods



and May each year. The peak water temperatures in both years (20°C in 1968, and 19°C in 1969) were not attained until early August. The temperature build-up to the summer maximum each year was slow.

DISCUSSION

(a) Maturation

Seasonal maturation cycles have been shown for many fish proteocephalids, including Proteocephalus filicollis (Hopkins 1959, Willemse 1968), in temperate climates. P. filicollis did not, however, mature seasonally in a Yorkshire pond (Chappell 1968) and a Glasgow canal (Section 2), gravid worms occurring throughout most of the year. Seasonal maturation of P. filicollis did not occur in this present pond survey (Fig. 4). The April to August 1968 period when gravid worms were seldom present, was not repeated in 1969. The high percentage of gravid worms during the 1968/69 winter indicates clearly that P. filicollis overwintered in the fish both as plerocercoids and adult worms. The presence of small plerocercoids and copepods in the fish intestines throughout the year suggests continuous recruitment, even in winter. As discussed elsewhere (Section 2) this continuous plerocercoid recruitment, which did not occur in the Lanarkshire pond (Hopkins 1959) may be at least partly responsible for the non-seasonal maturation of P. filicollis in the pond.

(b) Incidence

Neither the incidence (Fig. 1) nor the worm burden

of the fish samples (Fig. 3) varied seasonally; a situation not unexpected considering the non-seasonal maturation of P. filicollis in the pond. The continuous recruitment of plerocercoids and the high ratio of non-gravid to gravid worms (Fig. 4) indicates that considerable loss of non-gravid worms occurred. Thus, as discussed more fully in Section 2 changes in incidence can be related to changes in the relative rates of worm loss and recruitment. The collapse of the worm burden of the fish sample in August 1969 (Fig. 3) represented a 91% worm loss, neglecting probable worm recruitment. The extremely high July burden, and hence possible inter-worm competition for space and food, together with the general instability of P. filicollis (Walkey 1967, Section 1) were probably responsible for this exaggerated worm loss. The presence of gravid worms in most months of the year and the continuous plerocercoid recruitment together suggest the presence in the pond, as in the canal (Section 2), of an ever present procercoid pool in the copepod population. The dynamics of fish tapeworms in the intermediate copepod host is discussed in Sections 2 and 5 .

(c) Worm location in the stickleback intestine

1. Plerocercoids

Plerocercoids of Proteocephalus filicollis were virtually confined to the rectum of sticklebacks in a Lanarkshire pond (Hopkins 1959), and showed a seasonal distribution pattern between the rectum and the intestine anterior to the ileo-rectal valve in the Glasgow canal (Section 2). Plerocercoids rarely occurred in the rectum in the present study. The problem of the preferred site of plerocercoid attachment was discussed in Section 2 where it was suggested that the initial site of plerocercoid attachment was not necessarily permanent and that plerocercoids could migrate freely within the intestine.

2. Strobilate worms

Strobilate worms, as in all other sites studied, were virtually confined to the intestine anterior to the ileo-rectal valve, gravid worms being concentrated in the anterior regions. One gravid and one immature worm were noted attached in the rectum, the strobila of the former hanging free from the anus. As discussed in Section 2 , the gravid worm was probably in the process of being lost. Of the strobilate worms in the pond, 43.2% were attached in the anterior intestine (Fig. 5) compared with only 27.4% in the canal (Section 2). The majority (48.2%) of the

canal strobilate worms were attached in the mid-intestine.

(d) The onset of strobilation

It was calculated that strobilation was most likely to occur in the cnnal (see Section 2) when plerocercoids were 3.4 mm. Hopkins' (1959) and Chappell's (1969) estimates of initial strobilation length were 5 to 6 mm, and 1.3 to 2.9 mm respectively. It was argued in Section 2 that plerocercoid position was one of the factors involved in determining the onset of strobilation. Thus plerocercoids anterior to the ileo-rectal valve could strobilate, while those in the rectum developed as plerocercoids and could only strobilate after moving anterior to the valve. The concentration of plerocercoids in the rectum throughout most of the year perhaps explains why Hopkins' plerocercoids strobilated at a much larger size than did Chappell's whose plerocercoids were at all times concentrated anterior to the ileo-rectal valve. The intermediate length for strobilation in the canal site can be explained by the more or less equal distribution of plerocercoids between the rectum and intestine proper.

Since most plerocercoids in the present study of the Glasgow pond were found forward of the rectum one might expect that the initial length for plerocercoid

strobilation would certainly be less than 3.4 mm as noted in the canal. To test this hypothesis the mean length of strobilate worms with 5 or fewer segments was calculated, and found to be 3.35 mm (37 measured). Thus pond and canal plerocercoids are most likely to strobilate at the same length. However, as in the canal, the length of newly strobilate worms ranged from 1.08 mm to over 4 mm indicating that strobilation could occur over a range of plerocercoid lengths. In the same way it was noted in the pond, as in the canal, that in most samples there was a considerable length overlap between the longest plerocercoids and the shortest strobilate worms indicating again that no fixed length for plerocercoid strobilation exists. The average size of pond plerocercoids was 1.28 mm, only fractionally smaller than the 1.32 mm calculated for canal plerocercoids. Thus although strobilation of plerocercoids can occur at different lengths, there is no difference between the average plerocercoid length, nor the length at which strobilation is most likely to occur, in the pond and canal sites.

(e) <u>The relationship of worm length, segment number, and maturation</u>

As the length and segment number of strobilate worms

increased, so also did their maturity (Fig. 7 & 8). The length and segment number overlap between non-gravid and gravid worms is similar to that in the canal (Section 2). The shortest gravid worm in both sites was approximately 6 mm while no worms with less than 12 and 13 segments in the pond and canal respectively were gravid. Although there tended to be fewer really long gravid worms in the pond, the growth and development of adult P. filicollis in the two sites was similar.

(f) Worm distribution in the fish samples

Considering the similar incidence of Proteocephalus filicollis in both sexes and the relatively even spread of the infection throughout the various size groups of fish each month (especially during the period September 1968 to September 1969) (Fig. 10) it is reasonable to conclude that, as in the canal (Section 2) both sexes and all sizes (and ages) of sticklebacks are equally liable to proteocephalid infection.

(g) Future research into the biology of Proteocephalus filicollis

The straightforward seasonal incidence and maturation cycles of P. filicollis shown by Hopkins (1959) and

Willemse (1969) were not apparent in the Glasgow canal (Section 2), a Yorkshire pond (Chappell 1969) and the Glasgow pond. Laboratory studies (Sections 1&6) demonstrated the copepod species in which the procercoid of P. filicollis could develop and also the time for full procercoid development at summer temperatures. Detailed study of procercoid development at lower temperatures, together with intensive investigation of the development and longevity of the worm in the stickleback are required before any suggestions as to the factors determining the different incidence and maturation patterns of P. filicollis in the various sites can be put forward. The difficulty in keeping the worm in the fish host in the laboratory (Section 1) is the main obstacle to be overcome before the achievement of the above goal.

SUMMARY

1. The incidence, intensity of infection and development of Proteocephalus filicollis was studied by the monthly examination of approximately 60 Gasterosteus aculeatus in a Glasgow pond over a 2 year period.

2. No seasonal incidence nor maturation cycle occurred.

3. Both plerocercoids and strobilate worms were seldom found in the rectum and showed no seasonal spatial distribution patterns. Gravid worms were concentrated in the most anterior regions.

4. Gravid worms were generally longer and possessed more segments than non-gravid worms. Although the onset of strobilation is not apparently directly dependant on plerocercoid size, it was calculated that plerocercoids are most likely to strobilate when 3.4 mm.

5. The Proteocephalus filicollis infection was spread evenly throughout the fish samples.

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SECTION 4

Studies on Proteocephalus sp. from the Loch Lomond powan Coregonus lavaretus (L.) I. Worm identity and procercoid development in two species of copepod.

(with 6 figures in the text)

INDEX

INTRODUCTION

Copland (1957) reported the presence of Proteocephalus fallax (La Rue 1911) in the intestine of the powan Coregonus lavaretus in Loch Lomond. During a study of the seasonal dynamics of this worm in the powan (See Section 5) it was noted that mature and gravid worms appeared to be considerably larger and more robust than P. fallax as described by La Rue (1911). A study of the major diagnostic characters of the worms was therefore undertaken.

Since powan are neither predatory nor canibalistic (Slack, Gervers and Hamilton 1957) it was assumed that they became infected by eating infected copepods. Eggs were obtained from gravid worms and the development of the procercoid in two species of copepod is described.

MATERIALS AND METHODS

Using gill nets, powan Coregonus lavaretus were caught each month for a 2 year period (See Section 5). Immediately after landing, the fish were taken to the laboratory, killed with a blow to the head, and the gut from stomach to anus removed. The guts were then placed singly in covered petri dishes containing 2 ml of Hanks' and maintained at 4°C. Within 24 hours each intestine was slit longitudinally and the adult worms, found in summer, were washed in Hanks' saline and then allowed to relax until dead in tap water at 4°C. Worms were fixed in 10% formalin, stained with Grañacher's Borax Carmine (Gurr's Ltd., London), and differentiated in 1% HCl /70% ethanol. Worms were cut into convenient lengths before clearing in xylene and mounting in Canada balsam. The taxonomically important diagnostic features of the worms' anatomy were examined and measured with a camera lucida.

When gravid worms were placed in water thousands of eggs were released. Under negligible coverglass pressure the dimensions of the various membranes of the egg were measured with a camera lucida. The gross morphology of the egg was also examined.

Two species of copepod, Mesocyclops leuckarti (Claus)

and Diaptomus gracilis were collected from Loch Lomond on the 29th July 1969, by towing a zooplankton net behind a boat. The plankton was taken to the laboratory in a 9 litre plastic container containing loch water and kept at 15°C with its lid off. After 2 days a few hundred ml of the contents of the copepod container were poured through a 210 μ seive (Endecotts, London). The collected copepods were then washed into a shallow dish with just enough water to cover the bottom. With the aid of a pasteur pipette individual adult females of both species were picked out and placed in 125 mm diameter crystalising dishes. Two dishes of Mesocyclops leuckarti and two of Diaptomus gracilis were set up. Each dish was filled with Loch Lomond water and placed in the dark in an incubator at 15°C.

On the following day, the eggs, which had been maintained at 4°C for two days after release from the gravid worms, were added to each dish. The ratio of eggs to copepods was considerably greater than 1 : 1 to ensure a high percentage infection. After 4 h the contents of each dish were poured separately through a 210 μ seive which retained the copepods but not the eggs. The copepods were then washed into 4 clean 180 mm diameter crystalising dishes containing Loch Lomond water and returned

to the 15° C incubator. Every 2nd day the water in the dishes was drawn off with a pasteur pipette and replaced with fresh loch water, a few ml of hay infusion was added and dead copepods removed.

During the next 23 days copepods were examined for the presence of developing procercoids. Mesocyclops leuckarti was mounted under a coverslip for examination. By moving the coverslip the copepod could be held in any desired position so that any procercoids present in the haemocoel could be noted. The more delicate Diaptomus gracilis were placed on a slide in a drop of water which was then drawn away carefully with a pasteur pipette leaving the copepod held relatively firmly in a thin film of water. The copepod was then examined. The dimensions of the developing procercoids in situ were measured with a camera lucida. For detailed examination procercoids were disected from copepods in crayfish Ringer and mounted individually.

301 of the Mesocyclops leuckarti caught on the 29th of July were not exposed to the eggs in the laboratory and maintained under identical conditions to the experimental copepods. These control copepods were examined for the presence of procercoids on the 14th August.

RESULTS

(1) Identity of adult worm

As noted by Doby and Jarecka (1914) the classification of proteocephalids with less than one hundred testes is based on the number, relative size, absolute size, character, or presence or absence of only 5 major anatomical features of the worm and embryo. The relevant data on these features of the proteocephalid in powan are presented in Table I alongside those of Proteocephalus fallax as described by La Rue (1911). The two worms are similar in that they both possess a functional 5th sucker and a uterus with 6-8 lateral branches. The proteocephalid in powan, however, has considerably more testes than P. fallax and a smaller oncosphere. The ratio of the length of the cirrus pouch relative to the width of the proglottid of the powan proteocephalid never exceeded the lower limit of that in P. fallax. P. fallax is a "quite small indistinctly segmented cestode measuring up to 100 mm long"; the powan proteocephalid however has distinct segmentation and the length of 25 gravid worms ranged from 82 mm to 222mm (mean 141 mm).

(11) The structure of the egg

Using the terminology of Rybicka (1966) the outer

Table 1 The principal diagnostic characters of Proteocephalus fallax La Rue 1911 and Proteocephalus sp. a parasite of the powan Coregonus lavaretus in Loch Lomond.

TYPE LOCALITY	TYPE HOST	TAPEWORM	NUMBER OF TESTES	5th. SUCKER	UTERINE BRANCHES	CIRRUS POUCH/ SEGMENT RATIO	DIAMETER OF ONCOSPHERE
L.Lucerne Switzerland	Coregonus fera	Proteocephalus fallax	30-35	Functional	6-8	1/3-1/2	31 — 36µm
L.Lomond Scotland	Coregonus lavaretus	Proteocephalus sp.?	38-63	Functional	6-8	1/3	24 — 29µm

envelope of 17 powan proteocephalid eggs ranged from 115 µm to 290 µm with a mean of 183 µm. The embryophore is 41-46 µm in diameter mean 42 µm (10 measured).

The diameter of the oncosphere membrane surrounding the oncosphere itself was not measured. The oncosphere had an average diameter of 27 µm (10 measured) with a range from 24-29 µm. As indicated in.Figure.1 in many eggs, after only a short time in the water, the oncosphere, still surrounded by the oncospheral membrane, came to lie within the outer envelope, after escaping from the embryophore. Hook and body movement of such oncospheres occurred. Oncospheres still within the embryophore remained motionless.

(111) Procercoid development

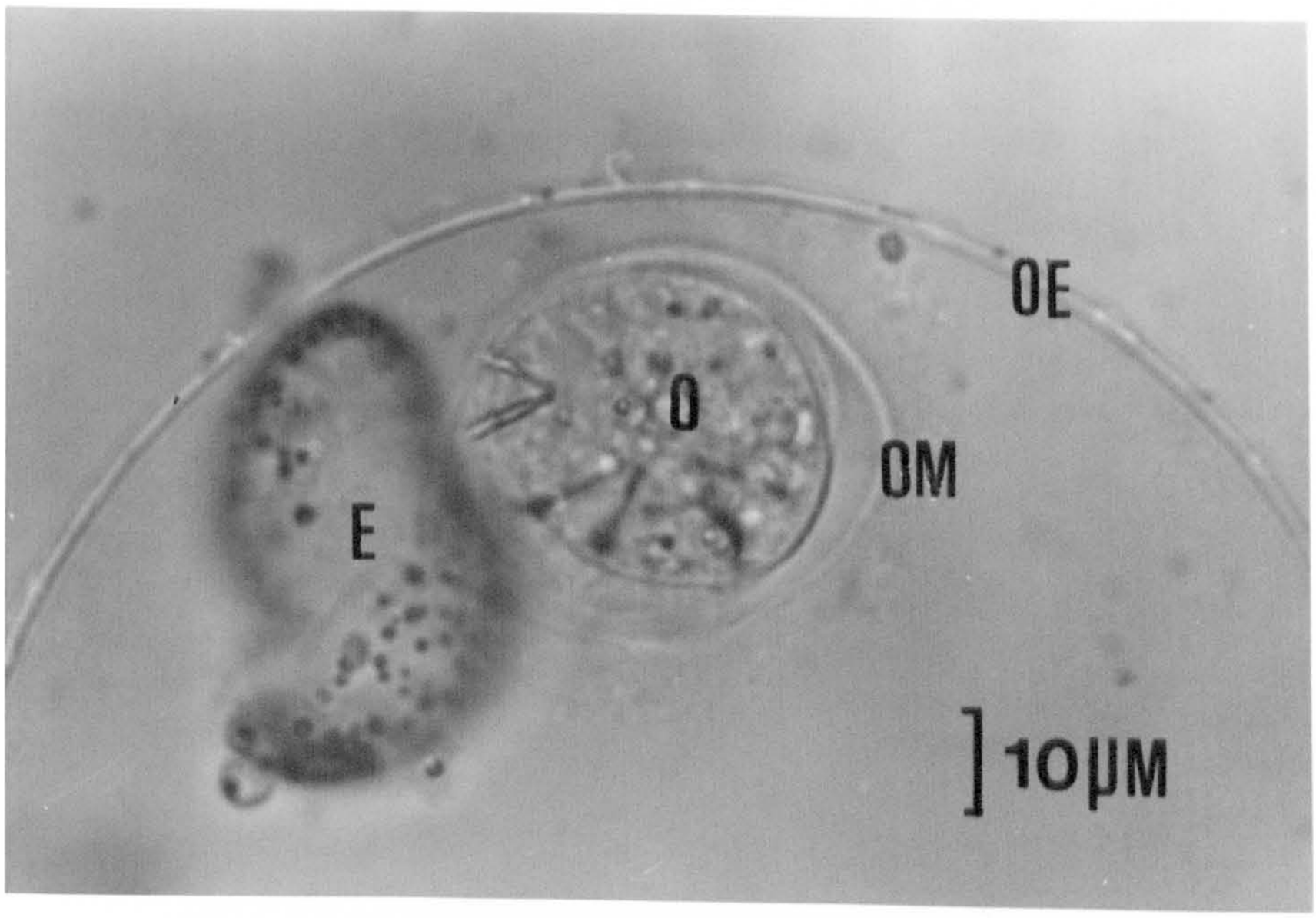
Both Mesocyclops leuckarti and Diaptomus gracilis as shown on Fig. 2 became infected and allowed procercoid development of the powan proteocephalid. After only 30 minutes the haemocoels of three M. leuckarti contained newly penetrated oncospheres. On the basis of worm length the pattern of growth of procercoids in both copepod species was similar. The scoleces of procercoids developing in M. leuckarti, but not in D. gracilis, were invaginated on the 19th day accounting for the shorter length of the former procercoids.

Figure 1

Infective egg of the powan proteocephalid, showing the oncosphere (O) free of the embryophore (E) yet still within the delicate oncospheral membrane (OM) and the outer envelope (OE).

Figure 3

Procercoids (P) of the powan proteocephalid after 19 days development at 15°C in the haemocoel of Diaptomus gracilis. The suckers (S) of one of the two procercoids are apparent. The procercoids are lying in the posterior region of the cephalothorax.


OE
O
OM
E
]10µM

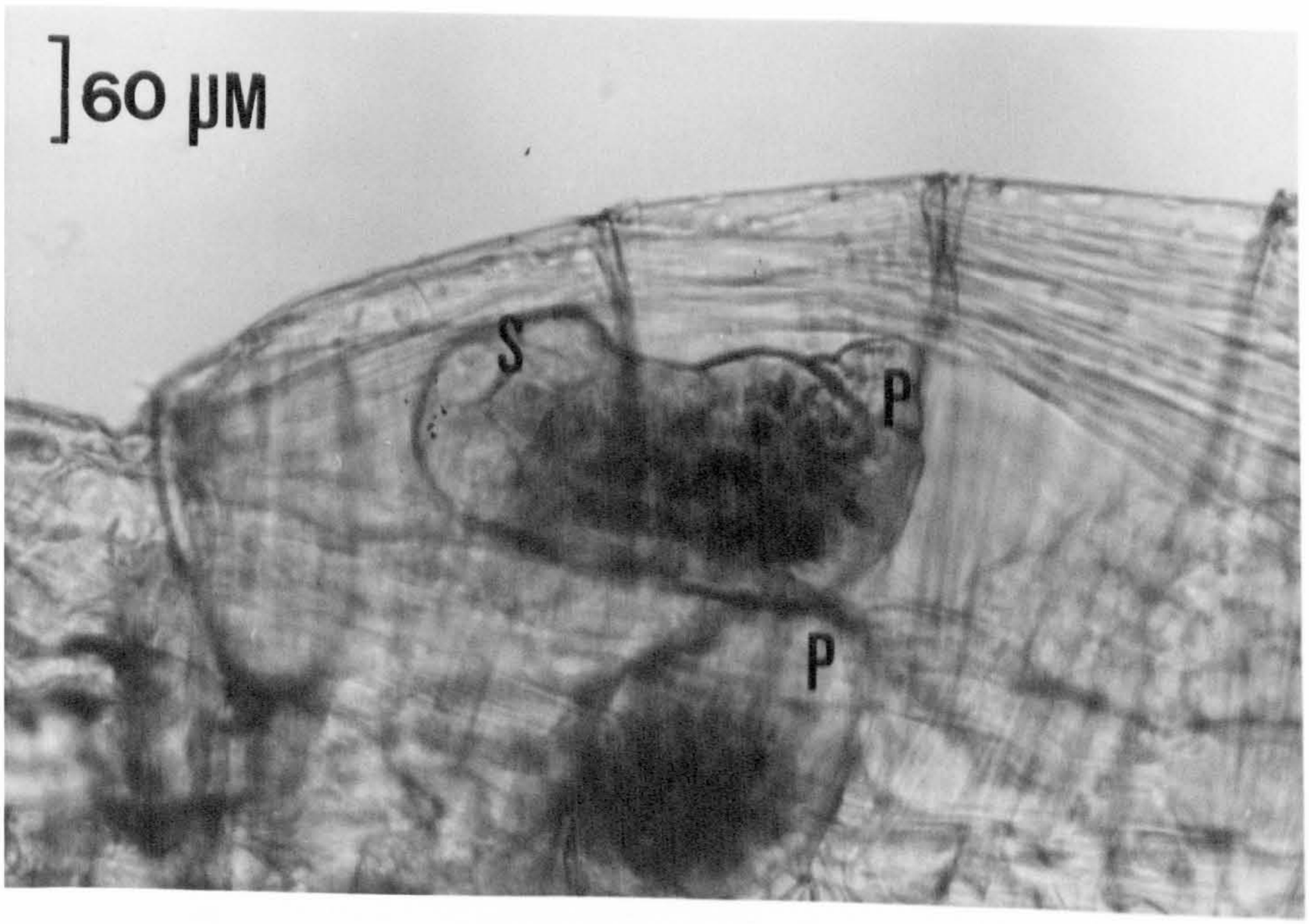
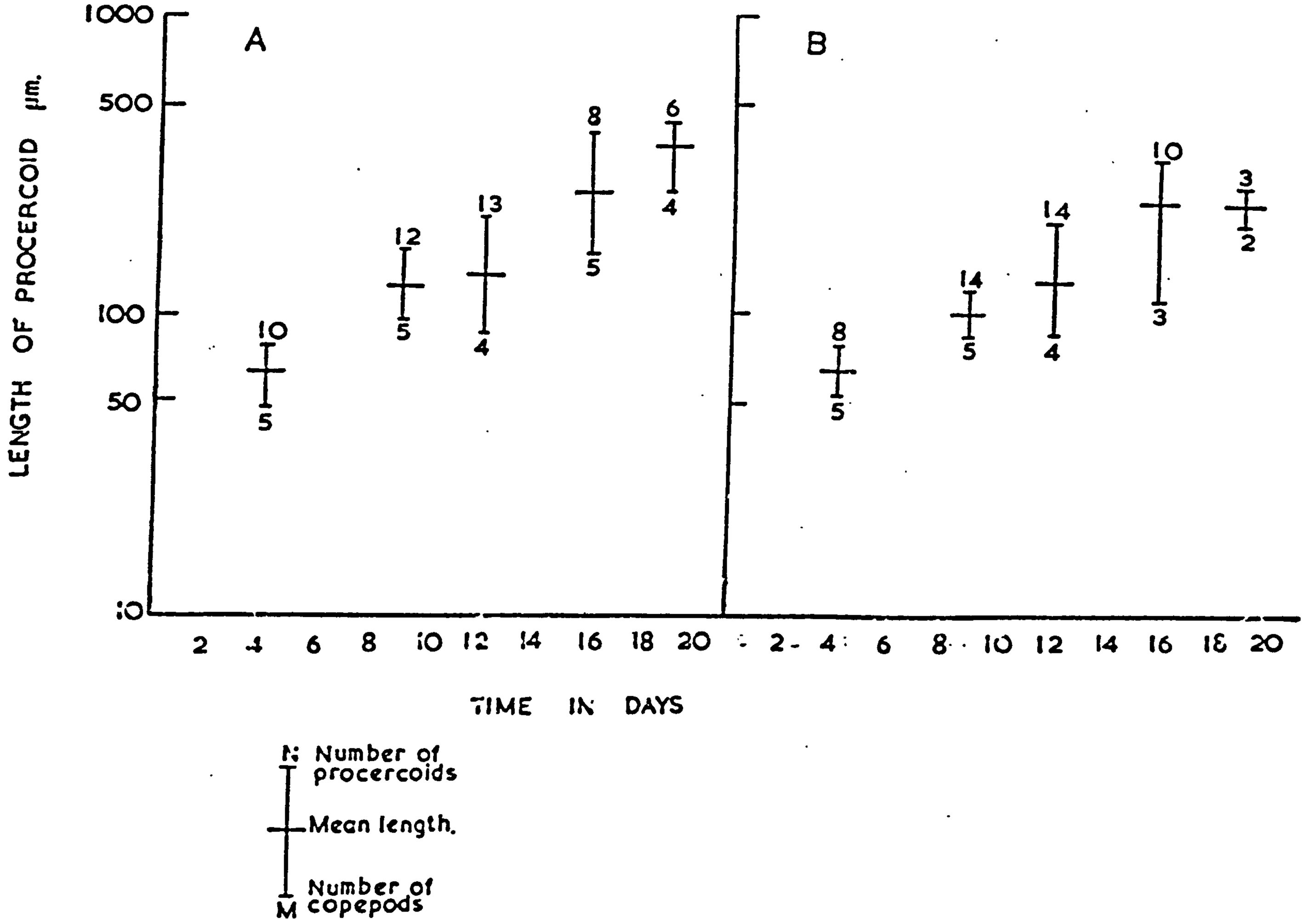

]60 µM
S
P
P

Fig 2 The growth of procercoids of Proteocephalus sp. from the powan Coregonus lavaretus in the copepods Diaptomus gracilis (A) and Mesocyclops leuckarti (B)



After penetration, the larva remained oncosphere-like for two days and then, as it grew, became vacuolate and amoeboid in appearance, taking on a more elongated than spherical form. Growth and elongation continued until the 16th day when a cercomer, which contained no larval hooks, was pinched off from the hind end. The cercomer was still attached to procercoids found on the 16th day. Such procercoids also possessed calcareous corpuscles and strong indications of sucker formation. By the 19th day the cercomers were all loose, yet still active, in the haemocoel, and the procercoids possessed fully developed suckers (see, Fig.3). The 5th sucker was also apparent at this stage. The scolex of procercoids developing in M. leuckarti had invaginated by the 19th day. A 23 day old fully developed procercoid within and free of the haemocoel of D. gracilis is shown on Figs. 4 and 5.

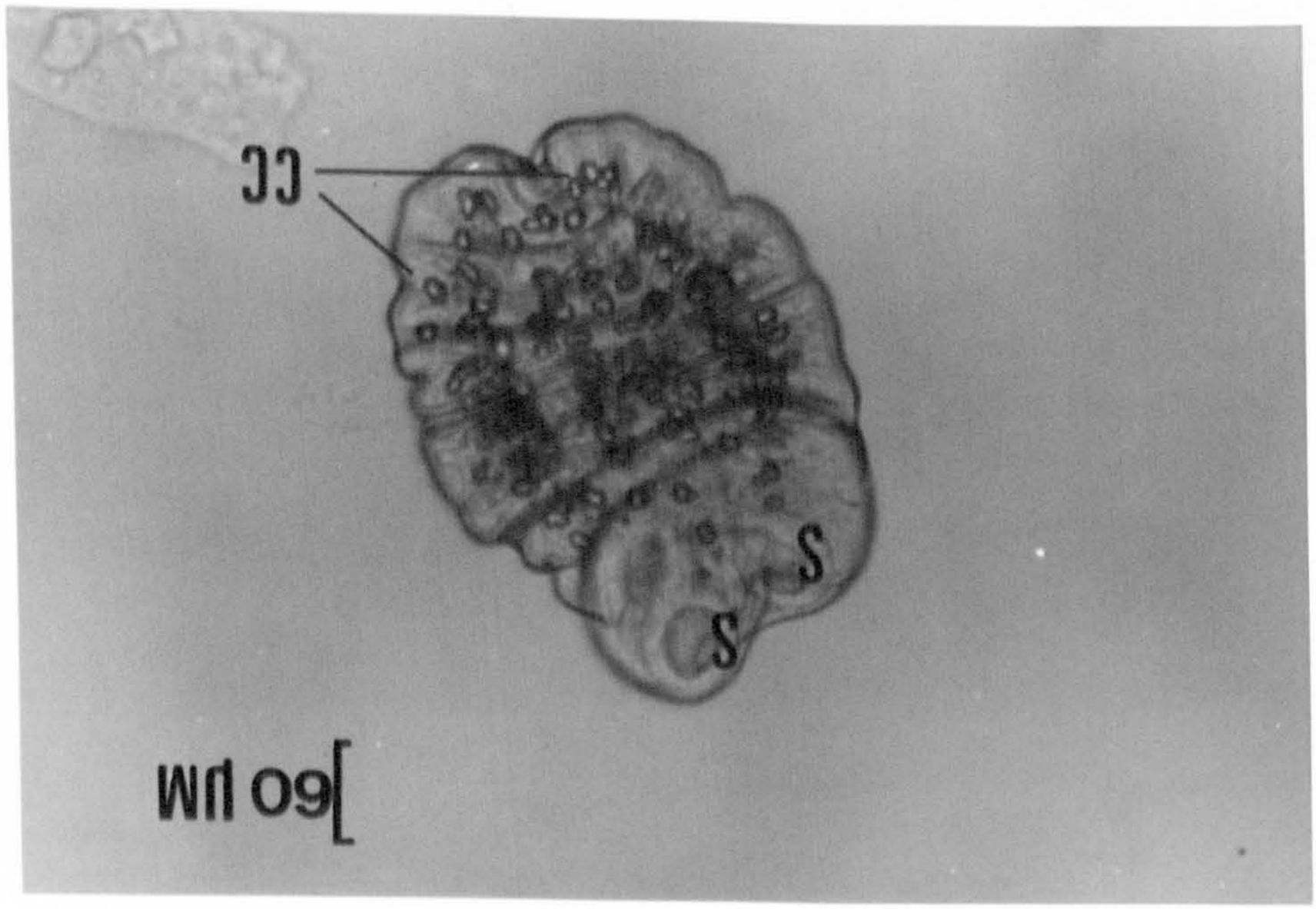
The following description of a fully developed procercoid of the powan proteocephalid is based on a procercoid removed from M. leuckarti in which it had been developing for 20 days at 20°C. With the scolex invaginated the worm measured 372 µm long by 122 µm broad. The 4 main suckers were approximately 45 µm in diameter with an internal diameter of approximately 20 µm. The outside diameter of the 5th sucker was 22 µm. The 70 calcareous

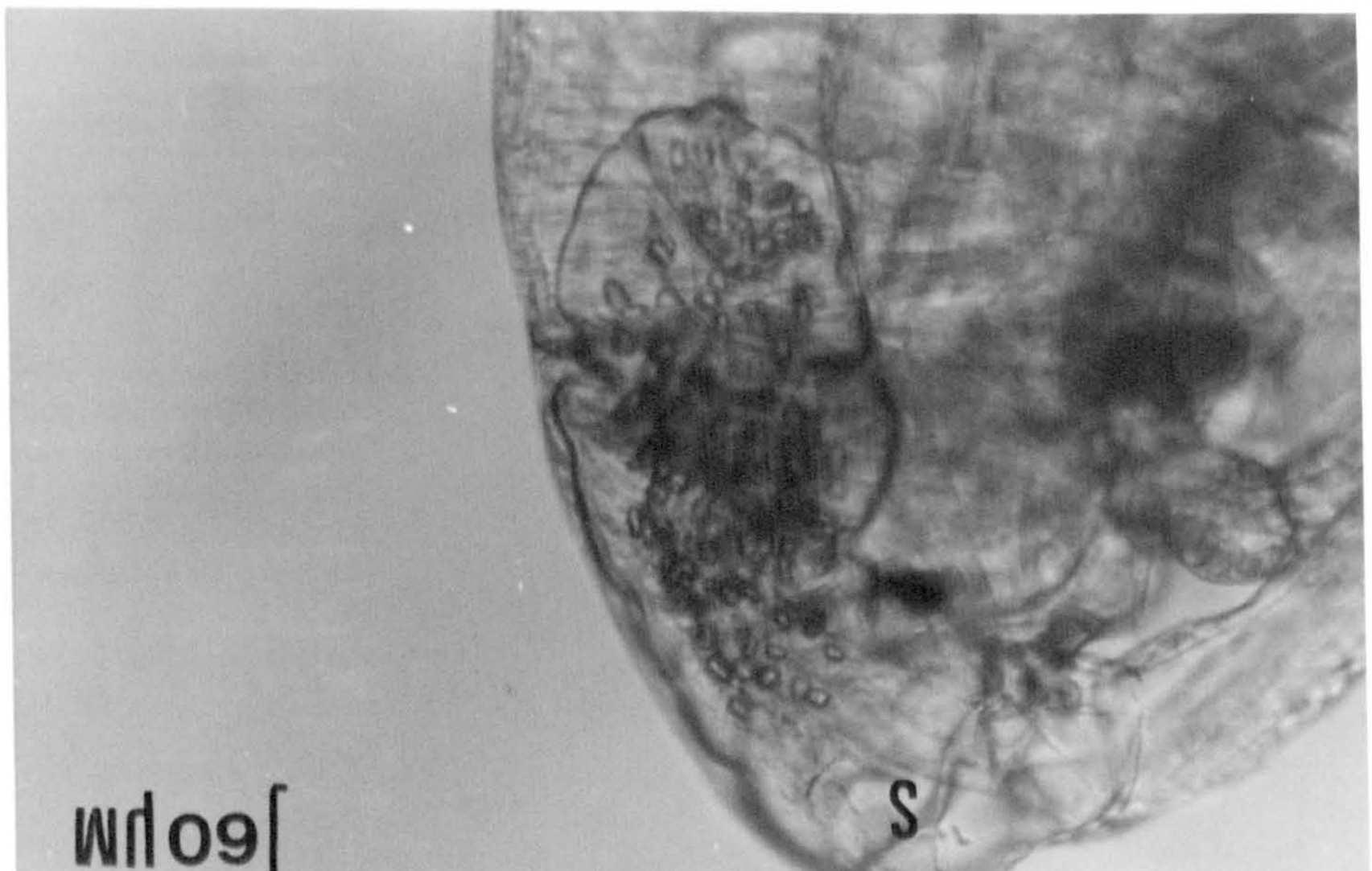
Figure 4

A procercoid of the powan proteocephalid after 23 days development at 15°C in the haemocoel of Diaptomus gracilis. The suckers (S) are indicated. The procercoid is lying in the anterior region of the copepod cephalothorax.

Figure 5

The 23 day old procercoid, shown in Figure 4, dissected free of the copepod. The suckers (S) and the calcareous corpuscles (CC) are apparent.


CC
S
S
60 µm

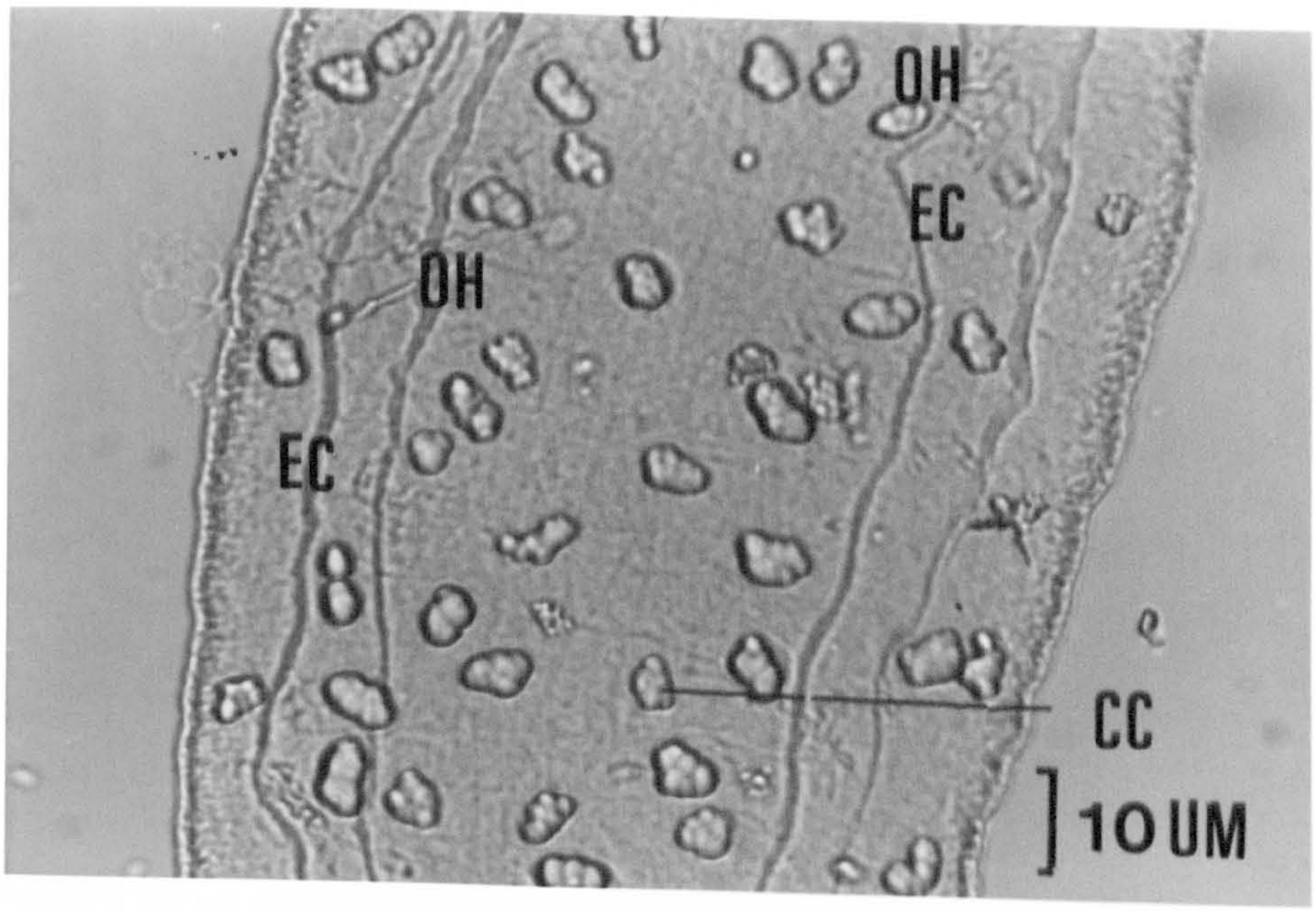

S
60µm

corpuscles present on this worm ranged from 0.49 to 1.2 μm in length. The excretory canals and apparently haphazardly distributed hooks of a procercoid from the haemocoel of M. leuckarti maintained for 21 days at 20°C. are shown in Fig. 6.

Only 11, of the 301 control M. leuckarti examined, were infected.

Figure 6

The mid-body of a procercoid of the powan proteocephalid dissected from the haemocoel of Mesocyclops leuckarti after 21 days development at 20°C. The excretory canals (EC), calcareous corpuscles (CC), and the scattered oncospheral hooks (OH) are indicated.


OH
EC
OH
EC
CC
10 UM

DISCUSSION

The proteocephalid infecting the powan, Coregonus lavaretus in Loch Lomond is very probably not Proteocephalus fallax as described by La Rue (1911), considering the major differences in their anatomy outlined in Table 1. The functional 5th sucker, the 6-8 lateral uterine pouches, together with the fact that the worm parasitised a coregonid fish probably led Copland (1957) to believe that the powan proteocephalid was Proteocephalus fallax. Using the information presented on Table I along with other data the writer, using the Keys of La Rue (1911) and Freze (1965), has not been able to identify the powan proteocephalid so it may be a new species. As yet, however, there is not enough information to justify naming it. Doby and Jarecka (1964) have recently studied the morphology of a proteocephalid from Coregonus fera in Lake Geneva but have been unable to ascribe it to any known species. It is clear, then, that the list of proteocephalid species infecting fish in Europe is not yet complete.

The egg of the powan proteocephalid conforms to the usual proteocephalid pattern (Rybicka 1966). The escape of the oncosphere from the confines of the embryophore has been discussed elsewhere and it is again emphasised that it is unlikely that the movement of the oncosphere within

the outer envelope could attract a copepod (See Section 1).

It is clear (Fig.2) that the two principal copepods in the Loch Lomond plancton (Chapman 1966) can both support growth and development of the procercoids of the powan proteocephalid. The pattern of procercoid development and the structure of the fully formed procercoids is similar to that of other proteocephalids (Doby and Jarecka 1966, Fischer 1967, Freeman 1964, Jarecka and Doby, 1965, Wagner 1954, and others).

Although under laboratory conditions Mesocyclops leuckarti and Diaptomus gracilis prove hosts of equal merit to the powan proteocephalid, it is uncertain if this is the case in Loch Lomond. In one copepod sample (see Section 5). 21% of 56 M. leuckarti were infected while no infection was found in 300 D. gracilis. In another sample one fully developed procercoid of the powan proteocephalid was found in a Diaptomus gracilis.

This difference between field and laboratory incidence may arise because D. gracilis were maintained, during exposure to proteocephalid eggs, relatively densely surrounded by eggs. Even under these conditions it was observed that eggs were not drawn in with the feeding currents of the copepod, but rejected to the side, although on occasions they must obviously have been ingested. To

what extent egg ingestion by D. gracilis occurs in the field remains unknown, but as D. gracilis is microphagus it probably plays a negligible part in the life-history of the powan proteocephalid in Loch Lomond. A detailed field study of the biology of the powan proteocephalid in Loch Lomond would be necessary to prove this.

SUMMARY

(1) Certain diagnostic features of a proteocephalid infecting the powan (Coregonus lavaretus) in Loch Lomond suggest that the worm is not Proteocephalus fallax (La Rue 1911) as stated by Copland (1957) but an unknown species.

(2) The structure of the egg and the development of the procercoid of the powan proteocephalid in two species of copepod Mesocyclops leuckarti and Diaptomus gracilis is described and discussed.

(3) The relative importance of the two copepod hosts in the ecology of the powan proteocephalid is discussed.

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SECTION 5

Studies on Proteocephalus sp. from the Loch Lomond powan Coregonus lavaretus (L.)

II Seasonal incidence and maturation.

(with 5 figures in the text)

INDEX

INTRODUCTION

Many parasites of fresh water fish living in temperate climates show definite seasonal incidence and maturation cycles. More specifically Connor (1953), Hopkins (1959), Grimaldi (1964) and Kennedy and Hine (1969) have described this phenomenon occuring in various proteocephalid tapeworms. Marked differences exist between the cycles outlined by the various authors with the result that a set generalised pattern describing proteocephalid incidence and maturation in fish of temperate waters cannot be formulated. To ameliorate this situation the author decided to study the variations in incidence and development of a species of Proteocephalus known to occur in the Loch Lomond powan, Coregonus lavaretus .

Proteocephalus fallax was reported by Copland (1957) to inhabit the intestine of powan. Subsequent studies (see Section 4) suggested that the species of Proteocephalus in powan is not Proteocephalus fallax but an as yet unidentified species. The pelagic copepods, Mesocyclops leuckarti (Claus) and Diaptomus gracilis have been shown, both in the laboratory and in Loch Lomond, to be suitable intermediate hosts for procercoid development of this species of Proteocephalus. There is no second inter-

mediate host, the powan acquiring the parasite by eating infected copepods. The powan feeds on plankton in the summer, but becomes benthophagous in winter (Slack, Gervers, and Hamilton 1957). This seasonal host dietary cycle might well be expected to enhance any existing temperature controlled cycle of proteocephalid incidence and maturation.

MATERIALS AND METHODS

From September 1967 until December 1969 gill nets were used to catch monthly samples of powan from Loch Lomond. The nets were always laid near the University Field Station 3 miles south of Rowardennan on the east side of the loch. The nets were laid at right angles to the shoreline in deep water from November until April or May, and in shallow water in summer and early autumn. The nets were lifted after 3 h.

Immediately after landing, the fish were taken to the laboratory, killed with a blow to the head, measured (from snout to the base of the tail fin), sexed, and the gut from stomach to anus removed. The guts were placed singly in covered petri dishes containing 2 ml. of Hanks' and kept at 4°C in order to inhibit bacterial decay. All guts were examined within 24 hours of placing at 4°C. By cutting the intestine transversely 4 times, 5 sections of approximately equal length were obtained: the pyloric region, immediately posterior to the stomach, the anterior intestine, the mid-intestine, the posterior intestine, and the rectum. Each section, the two most anterior of which bore numerous pyloric caecae, was slit longitudinally, the mucosa scanned with a disecting microscope (X25), and the number and condition of the worms attached in each region noted. Every

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month, a random selection of both plerocercoid and strobilate worms, if present, was removed from the mucosa, allowed to relax until dead in cold water, and measured. Worms were classed as plerocercoids (i.e. unsegmented worms), strobilate non-gravid worms, and gravid worms. In mid-summer the anterior half of the powan intestine was not cut into regions to avoid damaging the strobilae of gravid worms which stretched from the pyloric region to the mid-intestine.

The point of scolex attachment was regarded as the position of the worm, no matter its length, in the intestine. At certain times of the year newly acquired plerocercoids were found free in the gut lumen. Such unattached worms were included in the total worm population of the region in which they were found.

The contents of the stomach and intestine of each fish was examined to discover it's diet.

Plankton was collected at irregular intervals from November 1967 till December 1969 by towing a zooplankton net in the pelagic areas of the loch near where the fish were caught. The copepods Mesocyclops leuckarti and Diaptomus gracilis were examined for procercoids.

RESULTS

Details of the numbers of powan infected with *Proteocephalus* and of the numbers and condition of the worms found throughout the period September 1967 to December 1969 are shown in Table I.

(1) Incidence and mean worm burden

As shown in Fig.(1) the incidence of infection was very high, virtually 100% in summer, fell during autumn and early winter and reached its lowest level of 60% in January both years. The incidence increased throughout late winter and spring to reach 100% by early summer.

The mean worm burden (the average number of worms per infected fish) reached extremely high levels in late summer each year (Fig. 2). During autumn and early winter the mean worm burden fell and then rose slightly in late winter, spring and early summer. In mid-summer each year the burden fell temporarily before rising to the high levels of late summer. During this latter period the occasional fish with over 1,000 worms was encountered. Since the unusually high burden in January 1969 was due to the presence of two very heavily infected fish in the sample of 20, a more realistic figure for the January 1969 mean worm burden excluding these two fish has been shown in Table I and Fig.(1).

Table 1 Seasonal variation in incidence and development of Proteocephalus sp. in the L.Lomond Powan Coregonus lavaretus

Month and year	No.of Powan exam'd	Infected	Infection %	No.of worms found	Mean worm burden	Condition of worms plerocercoid	strobilate unripe	gravid
1967								
Sept	7	7	100	3278	468	3167	11	0
Oct	19	16	84	1316	82	1311	4	1
Nov	29	22	76	1569	71	1569	0	0
Dec	25	21	84	447	21	438	9	0
1968								
Jan	20	12	60	304	25	304	0	0
Feb	26	19	73	456	24	454	2	0
Mar	No sample taken							
Apr	19	16	84	184	12	182	2	0
May	19	17	89	436	26	406	0	30
June	14	14	100	798	57	544	251	3
July	12	12	100	352*	29*	33	146	173*
Aug	10	8	80	3173	397	2927	243	3
Sept	12	12	100	6134	511	6082	49	3
Oct	13	13	100	2072	159	2072	0	0
Nov	No sample taken							
Dec	18	14	77	189	14	189	0	0
1969								
Jan	20	12	60	763(148)	64 (15)	763	0	0
Feb	20	14	70	117	8	117	0	0
Mar	19	18	94	181	10	181	0	0
Apr	9	9	100	238	26	238	0	0
May	17	17	100	577	34	577	0	0
June	12	12	100	227	19	4	195	28
July	11	11	100	330*	30*	211	87	32*
Aug	12	12	100	2569*	214*	2434	116	19*
Sept	2	2	100	659	329	657	2	0
Oct	No sample taken							
Nov	No sample taken							
Dec	15	12	80	212	18	212	0	0

* Approximately due to difficulty in counting number of scolices of gravid worms in heavy infections

N.B. The total worm count and the mean worm burden for January 1969 excluding the two heavily infected fish are shown in parenthesis

Fig1 The incidence of infection of Proteocephalus sp. in Coregonus lavaretus in L.Lomond from September 1967 to Dec 1969

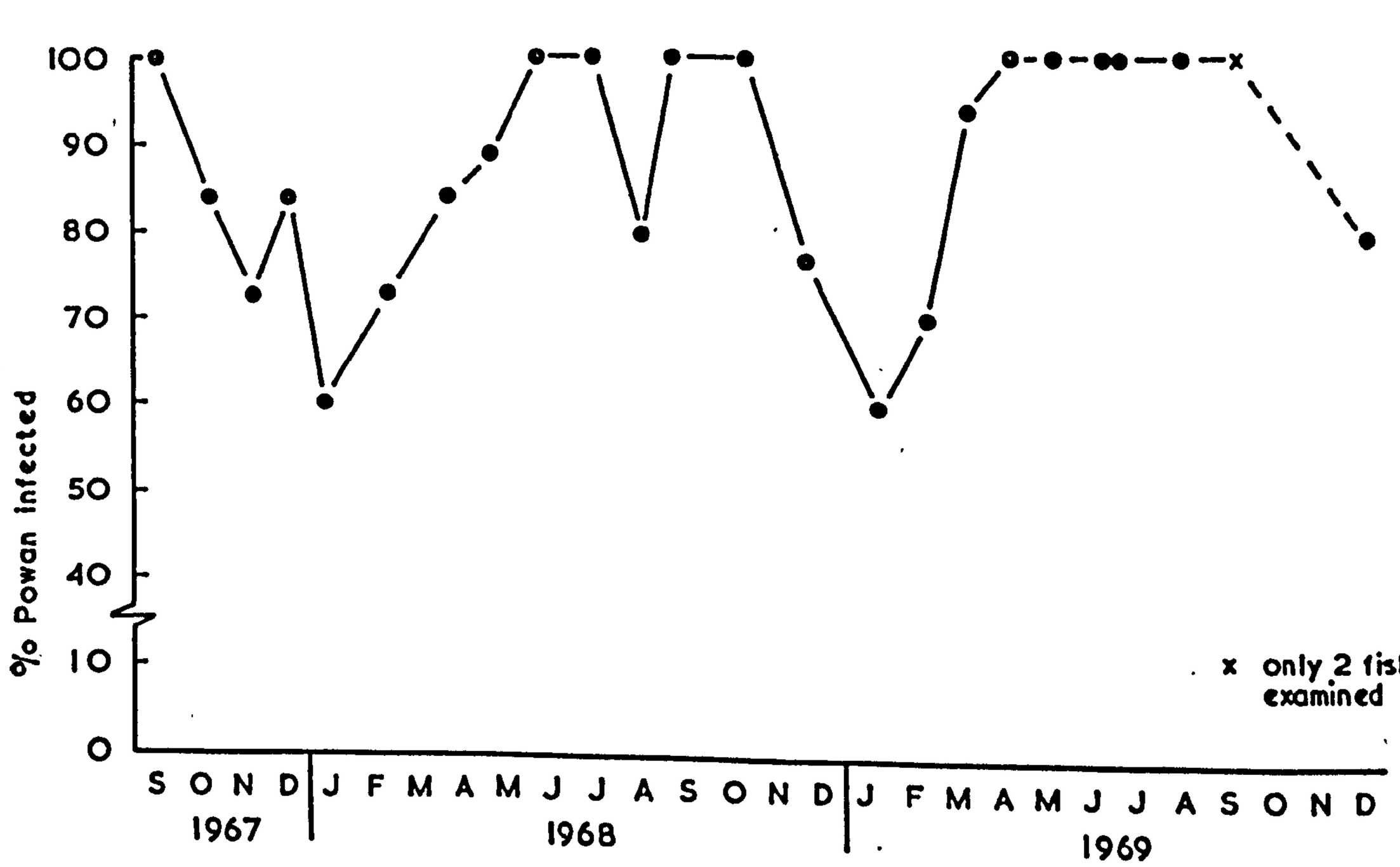
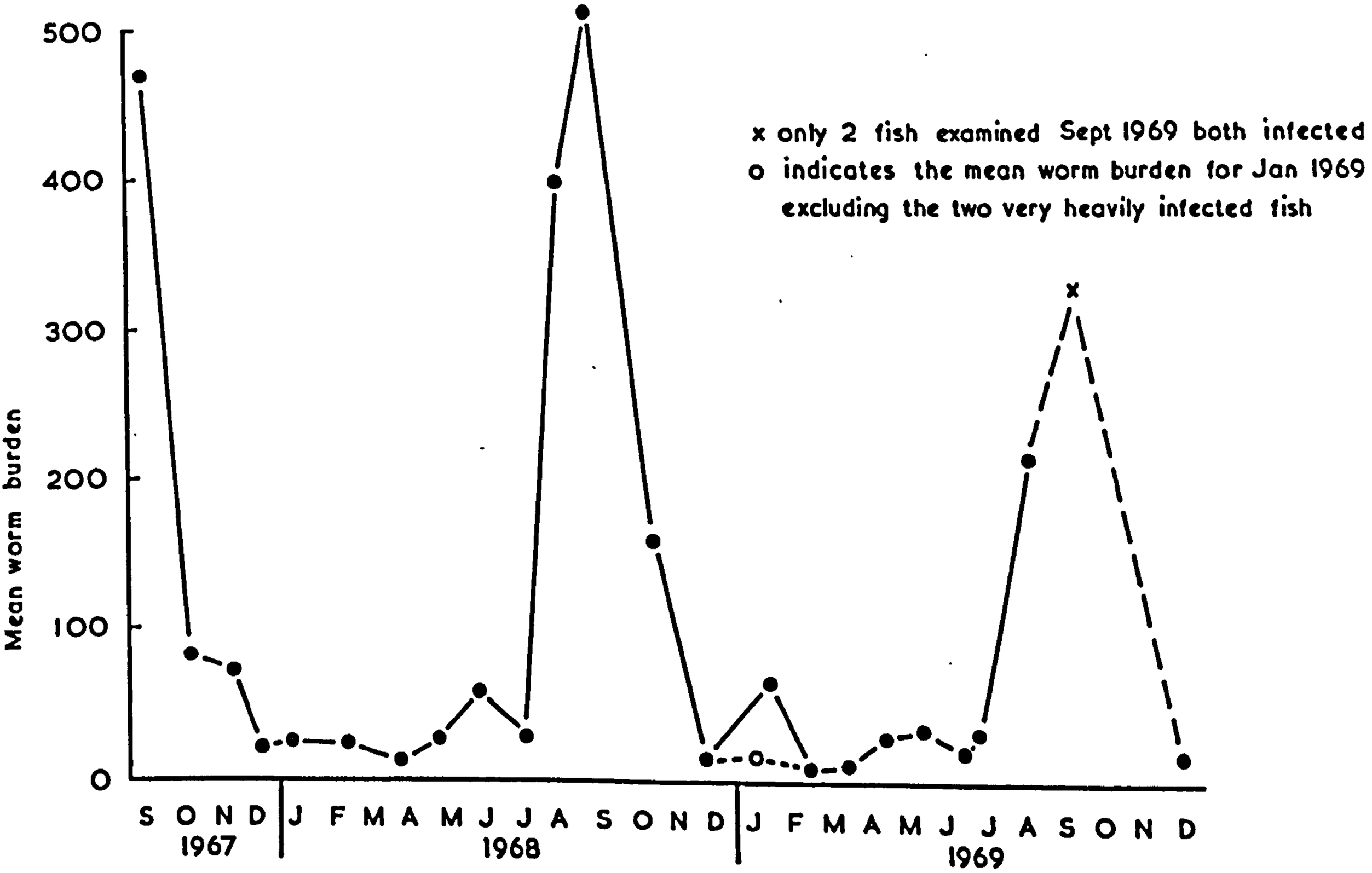
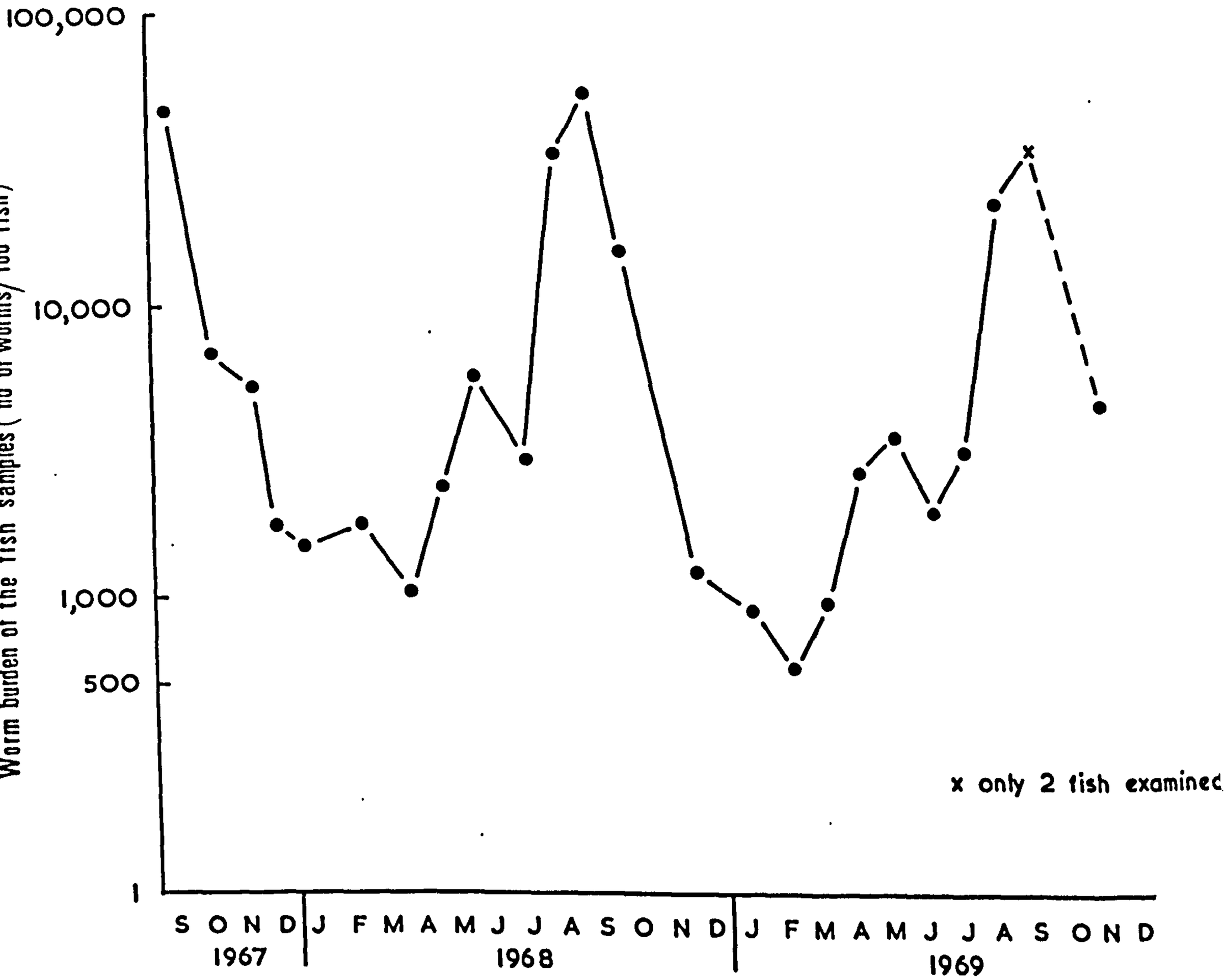


Fig 2 The mean worm burden of Proteocephalus sp. in the Powan Coregonus lavaretus



As shown in Fig (3) the worm burden of the fish sample (the product of the incidence and mean worm burden) reached its highest level in late summer, fell in autumn and early winter, and then rose in late winter, spring and early summer. The worm burden of the fish sample fell slightly in July 1968 and June 1969 before rising in late summer. The point of calculating the worm burden of the fish samples is explained in Section 2.

Fig 3 The worm burden of the samples of Coregonus lavaretus caught in Loch Lomond



(2) Development

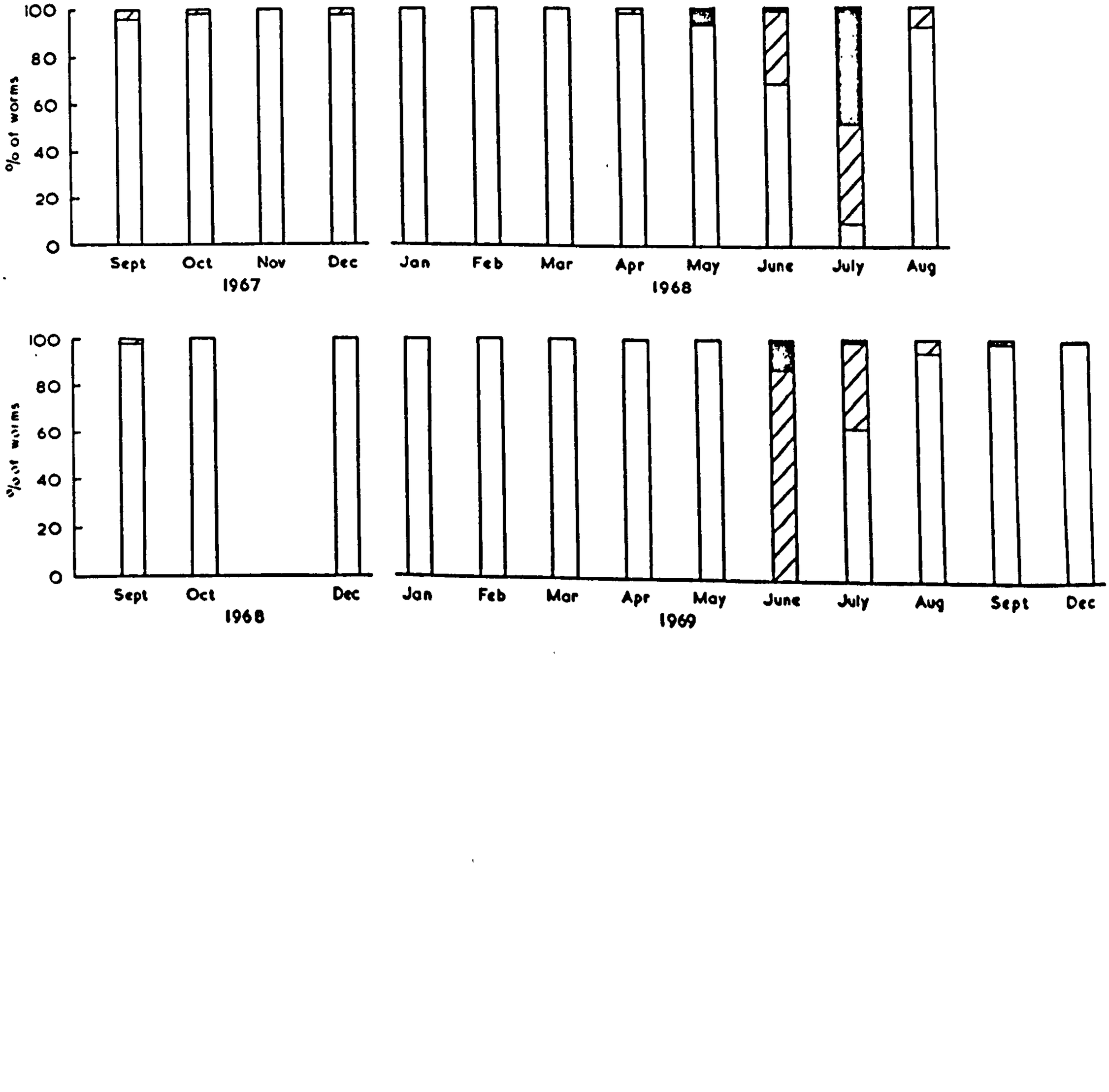
As is clear from Fig (4) for most of the year *Proteocephalus* occurs as a plerocercoid, strobilate non-gravid and gravid worms occur mainly in the summer. Gravid worms were most abundant in July 1968 and June 1969. The gravid worms noted in May 1968 were confined to 1 of 17 fish and contained only partially developed eggs. In July and August each year sections of gravid worms and almost complete worms were frequently found loose in the intestine.

(3) Worm Size

Plerocercoids were most commonly about 1 mm in length ranging from 0.36 mm to 11 mm, while strobilate worms ranged from 10-200 mm. Very small plerocercoids only 0.6 mm or less were common in September and October 1967, August and September 1968, and July and August 1969. Similar small worms were also encountered in April and May 1968 and May 1969. During the months February to May 1968, and March to May 1969 larger plerocercoids ranging from 2 mm to 8 mm were often noted. Of the strobilate worms, most non-gravid worms were less than 80 mm, while gravid worms normally measured between 100 and 160 mm. It should be stressed that the above details of worm lengths are based on measurements taken of a small number of worms randomly selected from each sample.

Fig 4. The development of Proteocephalus sp. in Coregonus lavaretus in L.Lomond

For each month the percentage of plerocercoids (clear), strobilate non-gravid worms (crosshatched) and gravid worms (black) is shown

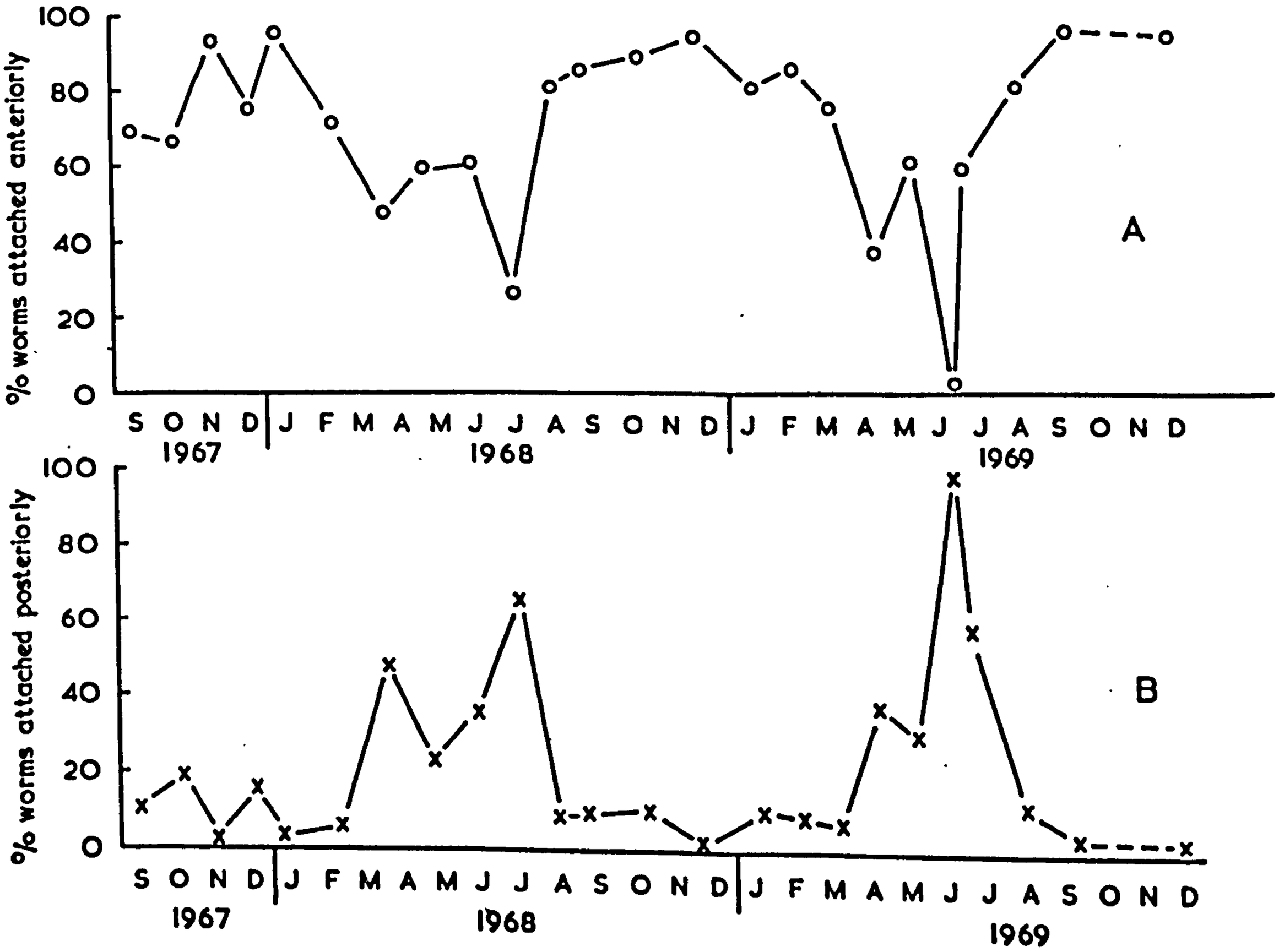


(4) Worm location in the host intestine

The percentage of all worms found each month in the anterior and pyloric gut regions combined and the posterior and rectal regions combined are shown in Fig.(5). Throughout the late summer, autumn, winter and early spring, the worm population was concentrated in the posterior gut regions. In April of both years, however, the percentage attached anteriorly increased, and then fell slightly before rising to a peak in July 1968 and June 1969. By August of each year, however, the majority of worms were again found in the posterior gut regions. Relatively few worms, frequently less than 10%, were recorded at any time of the year from the mid-intestinal region.

Strobilate non-gravid and gravid worms made up the bulk of the worm population attached anteriorly. Strobilate worms had their scolices firmly attached within pyloric caeca. No more than one worm per caecum was ever noted. Since no or very few strobilate worms occurred in April in each year, it is clear that the large percentage of worms attached anteriorly in this month were plerocercoids. Throughout autumn, winter and early spring, when most worms were attached posteriorly (Fig.5), the worm population was basically plerocercoids (Fig.4).

Fig 5 The percentage of Proteocephalus sp. attached in the pyloric and posterior regions (A) and the posterior and rectal regions combined (B) of the intestine of the Powan Coregonus lavaretus of L.Lomond



(5) The powan diet

From December 1967 to May 1968, and from December 1968 to April 1969, and in December 1969 the intestines of most fish were empty (Table II).

Although benthic material was present throughout the year it was almost completely replaced in summer by plankton. Copepods, principally Mesocyclops leuckarti were especially prominent in October and November 1967, August 1968, and July and August 1969 when hundreds of copepods were found in each intestine.

(6) Procercoid incidence and burden in copepods

Of the 931 Mesocyclops leuckarti from 5 plankton samples examined (Table III), 128 were found infected, all except one of which contained only a single procercoid of Proteocephalus. Identification was based on the presence of the apical 5th sucker. The incidence was much higher in winter than summer. At the same time as M. leuckarti were collected (Dec. 1969), 300 Diaptomus gracilis were caught and none was infected.

Table 2 Seasonal variations in the diet of the L.Lomond Powan (C.lavaretus)

YEAR	MONTH	NO. OF FISH			
		total no.	with empty guts	with ben-thos	with cope-pods
1	Sept	7	?	?	?
9	Oct	19	4	7	5
6	Nov	29	19	10	4
7	Dec	25	21	2	1
	Jan	20	15	5	0
	Feb	26	25	0	1
1	Mar	—	—	—	—
9	Apr	19	16	1	3
6	May	19	17	0	0
8	June	14	6	0	1
	July	12	4	0	1
	Aug	10	0	1	4

YEAR	MONTH	NO. OF FISH			
		total no.	with empty guts	with ben-thos	with cope-pods
1	Sept	12	0	0	2
9	Oct	13	1	1	1
6	Nov	—	—	—	—
8	Dec	14	11	1	0
	Jan	12	6	3	1
	Feb	14	13	1	0
1	Mar	18	16	2	0
9	Apr	9	5	1	2
6	May	17	2	9	2
9	June	12	0	0	0
	July	11	0	0	5
	Aug	12	0	0	6
	Sept	2	0	1	1
	Oct	—	—	—	—
	Nov	—	—	—	—
	Dec	15	13	2	0

Table 3 The incidence and worm burden of proteocephalid procercoids in the copepod Mesocyclops leuckarti in L. Lomond

Year and month	No. of copepods examined	infected	Infected %	Procercoid burden of infected copepods single	double
1967					
Nov	130	29	22.3	28	1
1968					
Dec	100	66	66.0	66	0
1969					
14 Aug[x]	301	11	3.6	11	0
28 Aug	344	10	2.9	10	0
Dec	56	12	21.4	12	0

[x] Collected 29th July 1969.

DISCUSSION

(a) Seasonal incidence and maturation

Strobilate non-gravid and gravid worms were found in powan during the summer, but from September until April or May of each year virtually only plerocercoids were present. Thus, like P.filicollis in Gasterosteus aculeatus (Hopkins 1959), P. stizostethi in Stizostedion vitreum vitreum (Connor 1953), and P. torulosus in Leuciscus leuciscus (Kennedy and Nine 1969), and other fresh water fish tapeworms in temperate climates, the species of Proteocephalus in powan matures seasonally resulting in a markedly seasonal cycle of incidence and maturation.

Strobilate worms in summer were, through fragmentation, virtually absent by autumn being replaced by vast numbers of young plerocercoids taken in during late summer and early autumn. This new plerocercoid population decreased in early winter (Table I, Figs. 2 & 3). The worms underwent little development during the winter. Renewed plerocercoid recruitment in late winter, spring and early summer seemed likely considering the increasing worm burden of the fish sample (Fig. 3). Although a little plerocercoid growth occurred in late winter and early spring, the main growth period was early summer with the development of long

strobilate worms.

(b) The adult worm

The scolex of strobilate worms in powan, was, like that of P. pollanicola in the pollan (Coregonus pollan) of Loch Neagh, as noted by Gresson & Corbett (1954), invariably firmly attached within a pyloric caecum. As no more than one worm per caecum was ever found it seems likely that the presence of a worm prevents the attachment of another within the same caecum. In mid-summer the anterior regions of the gut of infected fish was swollen by the strobilae but by September most strobilate worms had gone.

Kennedy (1969) and Kennedy & Hine (1969) argued that loss of adult Caryophyllaeus laticeps and Proteocephalus torulosus from dace (Leuciscus leuciscus) in the River Avon was due to a temperature dependent immune response against the worms, resulting in worm rejection in summer and immunity from reinfection until the return of low water temperatures in December. The loss of adult worms from powan, however,was closely followed, as in 1968, or accompanied by,as in 1969, reinfection with plerocercoids, thus rendering any suggestion of an immune rejection of adult Proteocephalus by powan untenable. The three-spined

stickleback (Gasterosteus aculeatus), according to Hopkins (1959) showed no indication of developing immunity against Proteocephalus filicollis.

(c) Worm location in the fish intestine

During the main infection period of late summer and autumn most plerocercoids became established in the posterior of the powan intestine (Fig.5).

The concentration of plerocercoids in the powan rectum and posterior intestine in winter, suggests that these regions are more favorable for worm survival, than are more anterior regions. The mucosa of the posterior intestine and rectum is ridged and generously supplied with villi among which most plerocercoids are found in winter. Thus the micromorphology of the hind intestine may make it more suitable for worm attachment and maintenance than do the anterior regions. The fact that strobilate worms attach themselves within the caeca and not to the smooth mucosa, suggests that the general mucosal surface in the anterior intestinal regions is unsuitable for permanent worm establishment.

Some evidence of anterior migration of plerocercoids to the anterior and pylonic regions of the intestine was noted in April and May of each year. A similar migration

pattern occurred in P. filicollis in G. aculeatus, plerocercoids being restricted to the rectum, while strobilate worms were attached just posterior to the stomach (Hopkins 1959). This phenomenon of helminth migration in fish intestine has also been noted by Chubb, Awachie, and Kennedy (1964) in acanthocephalan infections in brown trout.

Plerocercoids, especially in April and May, have been found attached within pyloric caeca, while strobilate worms have never been found attached anywhere but in caeca. It is possible that scolex attachment within a caecum by a growing plerocercoid is a prerequisite for the onset of strobilation.

(d) Plerocercoid loss from the fish intestine

Overall plerocercoid loss from the powan intestine was indicated by the decreasing incidence, mean worm burdens, and worm burden of the fish sample in autumn and early winter (Table I, Figs. (1), (2) & (3)]. Loss of immature worms from fish has been noted by other authors among whom Hopkins (1959) estimated that less than 1% of established P. filicollis plerocercoids survive to become gravid. Chubb, Awachie, and Kennedy (1964) and Hopkins (1959) have stressed that variations in incidence of a particular gut helminth in fish results from continual

variation in the relative rates of worm recruitment and loss. In autumn and winter worm loss from powan greatly exceeds recruitment, the worm burden in the fish sample falling, while in late spring, the balance swings the opposite way leading to an increased worm burden in the fish sample. The worm burdens in powan in the summer months are very low compared to those of late summer of the previous year indicating that, even excluding the net worm gain in late spring, the percentage worm survival to adulthood is very low. The gain of plerocercoids in late spring however ensures that sufficient worms were present to develop into egg producing adults in summer and so continue the cycle.

(e) The copepod host

The probable presence of many Proteocephalus eggs in the surface layers of Loch Lomond in August and September is probably responsible for the increased incidence levels of procercoids in copepods in November and December, as compared with August (Table III). Chapman (1966) has shown that Mesocyclops leuckarti present in Loch Lomond in November and December, overwinter, some copepods becoming dormant in the bottom mud, while a few remained in the plankton. The dormant copepods are believed to return to the plankton

in April. The late winter and spring plerocercoid recruitment can be explained by the probable ability of procercoids to overwinter on copepods. Thus the powan Proteocephalus in Loch Lomond probably overwinters both in the fish and the copepod host. Pecorini (1959) noted extremely low incidence of Proteocephalus spp. in Cyclops strenuus in Lake Maggiore throughout the winter. Higher incidence levels were found in summer, when as reported by Grimaldi (1964) adult Proteocephalus sp. were present in various whitefish (Coregonus spp.). Thus the ability of other species of Proteocephalus to overwinter as procercoids seems likely.

(f) Proteocephalus and the powan diet

The peak months of copepod ingestion by powan, as noted by Slack, Gervers and Hamilton (1957), were July, August and September, the same months in which it was found in this survey that powan intestines were full of copepods. Thus powan feed intensively on copepods during and after release of proteocephalid eggs into the water, resulting in extremely high worm burdens being recorded in powan in August and September.

SUMMARY

(1) The incidence and development of a species of Proteocephalus in the powan, Coregonus lavaretus, of Loch Lomond was studied over a 2 year period.

(2) An annual cycle of incidence and maturation was noted with gravid worms occuring in summer, and plerocercoids inhabiting the fish intestine throughout the rest of the year.

(3) No evidence of an immune rejection of adult worms in summer was noted, the loss of adults being followed immediately by the new generation of plerocercoids.

(4) Plerocercoids overwinter basically in the hind regions of the intestine, while strobilate worms in summer are found anteriorly attached. An anterior migration of plerocercoids in spring is suspected.

(5) The increasing worm population in late winter, spring and early summer, before the development of gravid worms in summer, suggests, along with other evidence, that procercoids overwinter in overwintering copepods.

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SECTION 6

Investigations into the host/parasite relationships of larval proteocephalids (Cestoda) and their copepod hosts.

INDEX

INTRODUCTION

During a study of the life histories of two proteocephalid fish tapeworms (see Sections 1 & 4), a number of instances were recorded in which procercoids failed to develop in laboratory infected copepods after an apparently normal gut penetration by the oncospheres. Lack of development and subsequent death of procercoids in the haemocoel of unsuitable copepod hosts has been noted by other authors. Guttowa (1961) has recorded death of young procercoids of the pseudophyllidean Diphyllobothrium latum in Cyclops strenuus strenuus and C. insignis. Freeman (1967) reported 'overcome' procercoids of Proteocephalus parallaeticus in the haemocoel of Cyclops bicuspidatus and C. vernalis. Cysticercoid development of the cyclophyllidean Drepanidotaenia lanceolata is completely inhibited in the haemocoel of 'accidental' copepod hosts (Kisielewska, 1959). The discovery of inhibited proteocephalid procercoid development in certain experimental copepod hosts stimulated the present investigation.

Infective proteocephalid eggs come into contact with copepods when gravid worms are lost from the definitive fish host. Normal procercoid development will result if a suitable species of copepod ingests the egg. In an unsuitable copepod, however, abnormal development will occur. In the former case all the conditions necessary for normal development have been

satisfied, while in the latter case at least one condition has not. Thus a study of the latter system may not only shed light on the onset and causes of inhibited procercoid development, but also on the conditions which determine host specificity in procercoid/copepod systems.

MATERIALS and METHODS

A zooplankton net was used to collect copepods from various sites. The net was towed behind a boat in Loch Lomond, while at various ponds and canals the net was hauled slowly through shallow areas. In all, five species of copepod were collected.

Copepods were generally maintained in their water of origin since this proved most suitable for prolonged survival.

Proteocephalid eggs were obtained from gravid worms. Three-spined sticklebacks, Gasterosteus aculeatus, in a canal and pond in Glasgow harboured a few gravid specimens of Proteocephalus filicollis in their intestines all year round. Gravid individuals of another species of Proteocephalus of as yet uncertain identity (see Section 4) were found during the summer in the intestine of the Loch Lomond powan Coregonus lavaretus. On transference of the worms from the fish intestine to fresh water, hundreds of infective eggs were released from each gravid proglottid.

To ensure high incidence levels in laboratory infections 30 - 300 copepods were exposed to a considerably greater number of eggs taken from a pool derived from many worms. Exposure of copepods to infective eggs was carried out in

10 cm diameter cystallising dishes filled to a depth of 1.5 cm with water. After two or three hours any remaining uneaten eggs were removed by passing the contents of the dish through a 210μ mesh sieve (Endecotts, London) which retains the copepods but not the eggs. The copepods were then washed with water collected from their site of origin into clean cystallising dishes and laid aside to await examination. As a rule, in order to eliminate any effect of sex or age of host on procercoid development, only adult female copepods were used in this series of experiments.

Individual copepods were mounted under a No. 2 coverslip for microscopic examination. By moving the coverslip the copepod could be rolled into any desired position so that the number and condition of worms present could be noted. Measurements of procercoids in situ were made with the aid of a camera lucida. To avoid examining the same copepod twice, all copepods examined were discarded.

The copepods used were Eucyclops serrulatus (Sars), E. serrulatus speratus (Lilljeborg), Cyclops albidus (Jurine), Mesocyclops leuckarti (Claus), and Diaptomus gracilis. Copepod identification was based on the key of Harding and Smith (1960), and verified, in the case of E. serrulatus speratus, by Mr Harris of the British Museum.

RESULTS

The fate of powan proteocephalid procercoids in E. serrulatus s.s.

Normal development of the powan proteocephalid procercoid occurs in M. leuckarti and D. gracilis. The former copepod, since it is more easily maintained in the laboratory, was used as a control in the two experiments. In both experiments, carried out in successive years, eggs fresh from gravid worms were added to two crystallising dishes one of which contained the controls, M. leuckarti, collected from Loch Lomond, while the other contained E. serrulatus s.s. from the Forth and Clyde Canal. The eggs were never removed from the control copepods in Expt. 1.

The results (Table I) show that growth of the powan proteocephalid procercoids in the haemocoel of E. serrulatus s.s. is very poor compared with growth in the control host. After 8 days at 15°C procercoids in M. leuckarti were 7 - 8 times the area of their still oncosphere-like counterparts in E. serrulatus ss. (Expt. 2). Initial incidence levels were high in both species of copepods. During the course of each experiment, however, the incidence in E. serrulatus ss. fell to a low level suggesting loss of the infection. This suggestion is supported by the occurrence of dead procercoids in the haemocoel of E. serrulatus ss. Dead larvae were

TABLE I THE DEVELOPMENT OF PROCERCOIDS OF THE POWAN PROTEOCEPHALID IN THE COPEPODS MESOCYCLOPS LEUCKARTI AND EUCYCLOPS SERRULATUS

EXPERIMENT 1 20°C

Time	MESOCYCLOPS LEUCKARTI					EUCYCLOPS SERRULATUS				
	No.of copepods exam'd	infected	No.of procercoids alive	dead	Mean size×range µm of live larvae	No.of copepods exam'd	infected	No.of procercoids alive	dead	Mean size×range µm of live larvae
14h	5	5	11	0	27×24 (24×24–29×24)	6	6	5	0	26×23 (24×22–28×26)
5d	6	5	13	0	64×50 (33×33–88×62)	10	3	4	3	28×25 (26×26–31×24)
10d	6	6	36	0	102×51 (20×29–50×39)	10	1	1	0	40×28

EXPERIMENT 2 15°C

1d	–	–	–	–	–	10	6	18	0	
3d	5	5	8	0	63×54 (56×47–71×59)	7	5	9	0	48×39 (39×37–81×37)
5d	5	5	8	0	100×79 (81×66–120×71)	5	5	13	1	44×40 (37×37–56×51)
6d	5	5	11	0	107×88 (81×81–145×118)	9	5	12	0	47×40 (37×37–56×51)
8d	3	3	8	0	174×91 (132×88–233×103)	20	11	12	5	49×43 (37×37–44×61)
11d	–	–	–	–	–	10	5	3	5	60×49 (54×47–69×44)

recognised either as shrunken, immobile, darkened larvae with splayed out oncospheral hooks, or as mere bundles of hooks devoid of any tissue remains of the procercoid. Dead procercoids were observed after 5 days in Expt. 1, but not until the 8th day in Expt. 2. Dead procercoids were never found in M. leuckarti; all the worms grew and developed normally. The continuous presence of eggs with the control M. leuckarti in Expt. 1 probably explains the increasing incidence of infection (i.e. the number of procercoids per infected copepod) which in turn might be responsible for the relatively low mean size of worm in the controls at 20°C compared with those at 15°C in Expt. 2.

The fate of procercoids of the powan proteocephalid in the haemocoel of Cyclops albidus

Samples of Cyclops albidus were collected periodically from Craigton Loch, eight miles North-West of Glasgow. As in the previous two experiments, M. leuckarti was used as a control. Exposure to eggs was carried out as previously. The copepods were kept at 20°C. The experiment was carried out twice and the results are presented in Table II.

The striking features of these results are the low incidence recorded for C. albidus and the high numbers of dead larvae (12 out of 14 in Expt. 3; 2 out of 6 in Expt. 4)

TABLE II THE DEVELOPMENT OF PROCERCOIDS OF THE POWAN PROTEOCEPHALID IN THE COPEPODS MESOCYCLOPS LEUCKARTI AND CYCLOPS ALBIDUS AT 20°C

EXPERIMENT 3

	MESOCYCLOPS LEUCKARTI				CYCLOPS ALBIDUS			
Time	No.of copepods exam'd	infected	No.of procercoids alive	dead	No.of copepods exam'd	infected	No.of procercoids alive	dead
1d	4	4	7	0	5	5	2	12

EXPERIMENT 4

1d	—	—	—	—	7	3	4	2
3d	5	5	8	0	8	2	0	2

found within 24 h in the haemocoel of those infected. Dead larvae were never observed in the haemocoel of M. leuckarti, in which, after 3 days in Expt. 4, the worms were growing.

The procercoids which did survive in C. albidus for three days showed no growth. Dead larvae appeared shrunken with splayed out hooks, or were often recognised by a bundle of disorganised hooks. No physical sign of a host response was noted.

The fate of Proteocephalus filicollis procercoids in the haemocoel of Cyclops albidus

Eucyclops serrulatus s/s. has been shown (see Section 1) to be a suitable intermediate host of P. filicollis, therefore this copepod was used as a control in the following two experiments. E. serrulatus s/s. were collected from the Forth and Clyde Canal and C. albidus from Craigton Loch. After exposure to the eggs the copepods in both experiments were kept at 20°C.

Procercoids in E. serrulatus s/s. appeared normal, and no dead procercoids were noted. In C. albidus, however, as shown in Table III, larvae of P. filicollis died a short time after penetrating the gut. In Expt. 6, after a total of 27 h, 11 of the 16 procercoids found were dead. In both experiments death was very rapid, in some copepods within

TABLE III THE DEVELOPMENT OF PROTEOCEPHALUS FILICOLLIS PROCERCOIDS IN THE COPEPODS EUCYCLOPS SERRULATUS AND CYCLOPS ALBIDUS AT 20°C

EXPERIMENT 5

Time	EUCYCLOPS SERRULATUS				CYCLOPS ALBIDUS			
	No. of copepods exam'd	infected	No. of procercoids alive	dead	No.of copepods exam'd	infected	No.of procercoids alive	dead
5h	3	2	6	0	3	1	1	0
10h	—	—	—	—	20	7	2	6

EXPERIMENT 6

6h	1	1	1	0	1	1	2	0
11h	3	2	4	0	4	3	1	2
22h	4	4	5	0	5	2	2	2
24h	—	—	—	—	2	1	0	2
27h	1	1	6	0	6	4	0	5

10 h of penetrating.

Six of the 11 dead procercoids found in Expt. 6 were enclosed in a sheath, and of the 5 live larvae observed, 2 were trapped in a matrix of filaments. One larva was observed for a considerable period of time and during this period it escaped from its entanglements only to be trapped again a few minutes later.

The fate of P. filicollis procercoids in the haemocoel of Eucyclops serrulatus speratus

Eucyclops serrulatus speratus is, on the basis of the length/breadth ratio of the furcal ramus, morphologically distinct from E. serrulatus s.s. On discovery of this copepod in a region of the Forth and Clyde Canal at Clydebank it was decided to test its suitabiliby as a host for the procercoid of P. filicollis, using E. serrulatus s.s. as a control. The incidence, as shown in Table IV, was high initially in both E. serrulatus s.s. and E. serrulatus speratus, but fell off gradually in E. serrulatus speratus until by day 7 only 1 of the 16 examined was infected. As only two dead larvae were observed in E. serrulatus speratus during the experiment it is impossible to conclude that the fall off in incidence

TABLE IV THE DEVELOPMENT OF PROTEOCEPHALUS FILICOLLIS PROCERCOIDS IN THE COPEPODS EUCYCLOPS SERRULATUS AND EUCYCLOPS SERRULATUS SPERATUS

EXPERIMENT 7

Time	EUCYCLOPS SERRULATUS					EUCYCLOPS SERRULATUS SPERATUS				
	No.of copepods exam'd	infected	No.of procercoids alive	dead	Mean size × range µm of live larvae	No.of copepods exam'd	infected	No.of procercoids alive	dead	Mean size × range µm of live larvae
14h	7	6	22	0		8	7	21	0	
24h	5	5	17	0		8	8	38	1	
40h	6	6	25	0	47x37 (39x34—56x39)	9	6	15	0	54x44 (42x34—61x49)
4d	6	6	20	0	53x 42 (42x34—69x54)	12	6	10	1	68x58 (49x44—81x76)
7d	6	6	20	0	71x 59 (51x47—98x71)	16	1	3	0	131x109 (120x98—142x108

was due to procercoid death. The few larvae which remained in E. serrulatus speratus grew as well as, if not better than, the control infections. Thus the fate of P. filicollis larvae in the haemocoel of E. serrulatus speratus is enigmatic. On the one hand dead larvae were found and the incicence dropped, while on the other hand the surviving larvae grew well. To try to solve the problem the experiment was redesigned.

Control and experimental copepods were maintained at 15°C prior to infection. After three hours exposure to eggs at 15°C, the controls (E. serrulatus s.s.) and experimental copepods (E. serrulatus speratus) were divided equally into two sets of control and experimental copepods. One set of each were maintained at 19°C while the other set were maintained at 11°C in Expt. 8, while in the repeat experiment, Expt. 9, done a year later, the copepods, both controls and experimentals, were kept at 19°C and 8.5°C. Copepods were periodically examined to judge the progress of the infection. The results of Expts. 8 and 9 are shown in Tables Vand VI respectively. Incidence levels remained relatively steady and no procercoid deaths were recorded in E. serrulatus s.s. at either temperature. However, a gradual fall off in incidence was noted in E. serrulatus speratus at either temperature.

TABLE V THE DEVELOPMENT OF PROTEOCEPHALUS FILICOLLIS PROCERCOIDS IN THE COPEPODS EUCYCLOPS SERRULATUS AND EUCYCLOPS SERRULATUS SPERATUS AT 19°C AND 11°C

EXPERIMENT 8

Time	EUCYCLOPS SERRULATUS 19°C					EUCYCLOPS SERRULATUS SPERATUS 19°C				
	No.of copepods exam'd	infected	No.of procercoids alive	dead	Mean size x range µm of live larvae	No.of copepods exam'd	infected	No.of procercoids alive	dead	Mean size x range µm of live larvae
1d	14	9	16	0	37x33 (32x29–39x39)	10	7	10	3	35x30 (29x26–47x34)
2d	—	—	—	—	—	17	7	19	5	51x37 (39x32–56x47)
4d	10	6	13	0	57x42 (39x37–71x49)	18	2	7	1	60x45 (42x27–76x53)
	EUCYCLOPS SERRULATUS 11°C					EUCYCLOPS SERRULATUS SPERATUS 11°C				
1d	7	7	13	0	42x35 (32x29–64x37)	9	8	22	1	42x33 (34x29--49x44)
2d	—	—	—	—	—	16	13	23	0	39x32 (34x20–42x42)
4d	8	7	10	0	42x34 (37x32–47x34)	12	8	15	3	42x35 (38x34–47x39)
6d	6	4	6	0	48x42 (39x39–56x51)	13	5	10	1	53x41 (42x29–61x51)

TABLE VI THE DEVELOPMENT OF PROTEOCEPHALUS FILICOLLIS PROCERCOIDS IN THE COPEPODS EUCYCLOPS SERRULATUS AND EUCYCLOPS SERRULATUS SPERATUS AT 19°C AND 8·5°C

EXPERIMENT 9

Time	EUCYCLOPS SERRULATUS 19°C					EUCYCLOPS SERRULATUS SPERATUS 19°C				
	No.of copepods exam'd	No.of copepods infected	No.of procercoids alive	No.of procercoids dead	Mean size x range µm of live larvae	No.of copepods exam'd	No.of copepods infected	No.of procercoids alive	No.of procercoids dead	Mean size x range µm of live larvae
1d	5	5	8	0		12	7	7	3	
2d	—	—	—	—	—	12	4	2	4	56x37 (51x37–61x37)
3d	7	5	8	0	59x46 (49x37–78x54)	16	2	4	2	56x51 (49x44–61x61)
	EUCYCLOPS SERRULATUS 8.5°C					EUCYCLOPS SERRULATUS SPERATUS 8.5°C				
1d	—	—	—	—	—	12	8	13	0	
2d	—	—	—	—	—	16	11	13	0	
3d	9	5	8	0	48x39 (37x37–56x44)	20	8	12	0	49x39 (42x34–59x42)
8d	10	3	3	0	59x43 (51x42–69x44)	12	5	2	5	59x47 (54x44–64x49)

Not all P. filicollis procercoids died in the haemocoel of E. serrulatus speratus. Those that did survive, as in Expt. 7, grew and developed as well as the controls. Generally speaking healthy growing procercoids were found in multiple infections of a few copepods. However 6 out of the 28 dead oncospheres found in the two experiments occurred in copepods which harboured a number of developing procercoids.

The onset of death of P. filicollis larvae in the haemocoel of E. serrulatus speratus

In Expt. 7, 87 live procercoids were observed in the haemocoel of E. serrulatus speratus. 25 procercoids were trapped by similar sheaths to those found enclosing P. filicollis larvae in C. albidus. Some procercoids were held tightly against the dorsal wall of the thorax, while others were trapped in tissue on the outside of the copepod gut. The majority of trapped larvae were located in the posterior section of the cephalothorax. The two dead larvae in this experiment were devoid of enclosing sheaths.

Of the 164 live procercoids of P. filicollis in E. serrulatus speratus in the latter two experiments, thirty seven were invested with a sheath. 11 of the 28 dead procercoids also possessed sheaths, the remainder being free

and shrunken with splayed out hooks.

No procercoid, alive or dead, >44 µm. long, was observed enclosed in a sheath. All larvae, >44 µm. long, were developing as well as the controls. One dead procercoid, 19 µm. in diameter, was enclosed in a sheath 85 µm. long by 49 µm. broad.

DISCUSSION

Either one of at least two possible fates await a proteocephalid oncosphere penetrating through the gut to the haemocoel of an unsuitable copepod host. Procercoids of the powan proteocephalid grew little if at all and subsequently died in the copepods E. serrulatus s.s. and C. albidus. Procercoids of P. filicollis also died in the copepods C. albidus and E. serrulatus speratus but there is good evidence in these cases that death is accompanied and assisted by the formation of an apparently temporary sheath around the invading larva.

Procercoid death without sheath formation has been encountered by other authors. Guttowa (1961) found that poorly developed procercoids of the pseudophylidean Diphyllobothrium latum became vacuolated, developed vesicles, shrank and died leaving a bundle of hooks in the body cavities of the unsuitable copepods C. strenuus strenuus and C. insignis.

Likewise Kisielewska (1959) has shown the pattern of the onset of death to be similar for cercocysts of the cyclophyllidean Drepanidotainia lanceolata in unsuitable copepod hosts. Neither author mentioned any intervention of amoebocytes in the process of procercoid death.

Actual vesicle formation was not observed, although the powan procercoids in C. albidus and E. serrulatus did appear pale and vacuolated. It is considered that the onset of death of the powan procercoid in these two unsuitable copepods followed an essentially similar pattern to that described for D. latum, by Guttowa (1961), in unsuitable copepods.

Death overtook powan proteocephalid procercoids fairly rapidly in the haemocoel of C. albidus, while the same larvae survived much longer and grew a little before death in E. serrulatus s.s. On this basis it can reasonably be suggested that the latter host is nearer to being a suitable host for the powan procercoid than is the former. Since there was no evidence of a cellular response by these two species of copepods, the factors determining the length of life of procercoids must lie in the contents of the haemocoel, viz. the haemolymph.

At least three hypotheses can be forwarded to explain the causes of death of the powan procercoids in these copepods. Firstly the haemolymph may be lacking in some

particular substance required for survival and growth of these procercoids. This could well explain the early death of the procercoids in C. albidus. A substance in short supply, however, could allow survival for some time and then, when the stocks of this substance were depleted, death of the procercoids would naturally follow. Such a situation would nicely explain the slow onset of death of procercoids in E. serrulatus s.s.

The second possibility is that the haemolymph is in some way toxic to the invading larvae. Toxicity may take the form of unsuitable osmatic gradients between procercoid and host. The pH of the haemolymph may even be unsuitable to the development of the larva. The presence, however, of a substance actually toxic to the larva does however seem less likely.

Thirdly, however, a subtle inbalance in the close delicate physiological links between host and parasite might exist in unsuitable copepod hosts.

Studies on the active absorption of amino acids by adult Hymenolepis diminuta in vitro, led Hopkins & Callow, (1965) to conclude that their 'observations implied that the concentration of amino acids in the tapeworm, and hence ability to grow, is greatly affected by the relative concentrations of amino acids in the environment'. The ratio of amino acids in the haemolymph of copepods varies from species

to species (Guttowa, 1970). Although it has not been shown, it seems probable that tapeworm procercoids will absorb amino acids in a similar way to adult tapeworms and that competitive inhibition will exist between animo acids utilising the same "carrier" system. Thus it may be the case that the molar ratios of amino acids in the haemolymph of an unsuitable copepod hœst may lead to unsuitable molar ratios of amino acids in the procercoid's own amino acid pool leading to negligible growth and subsequent death of the procercoids.

The formation of a sheath of material, considered to be made up of cells, accompanied or assisted the death of P. filicollis procercoids in the copepods E. serrulatus speratus and Cyclops albidus. This phenomenon was not observed by other authors in connection with procercoid death in unsuitable hosts.

A cellular response, against invading parasites, has however been noted in other crustaceans and insects. Hynes & Nicholas, (1958) have shown that acanthors of Polymorphus minutus become enclosed in a thin layer of host cells after penetrating an amphipod species different from that from which they were derived. This was followed by melanin deposition in the parasite and its death. With reference to insects (Salt, 1963) categorically states "that the blood

cells of many insects react to form capsules about living insect parasites cannot now be reasonable denied". Indeed with especial reference to tapeworm larvae in insect hosts, Chen (1943) has shown that cysticercoids of Dipylidium caninum in the flea Ctenocephalides felis became surrounded by blood cells and that completely encapsulated cysticercoids were invariably destroyed. In a brief survey, Salt (1963) concludes that defence reaction to metazoan parasites observed in crustacea are essentially very similar to those observed in insects.

From the author's own observation of ensheathed larvae the conclusion has been reached, although definite evidence is lacking, that the sheaths around the living and dead procercoids are made up of host blood cells which have been attracted towards the parasite. Cuenot (1895) has reported a similar reaction towards injected foreign material and helminth parasites in the decapod Astacus. Large particles of carmine injected into the haemocoel of Astacus attracted amoebocytes which became concentrated over the surfaces of the particles. He also found large numbers of dead trematodes, Distomum cirrigerum, encapsulated everywhere in the animal and verified that each parasite was surrounded by a thick "muff" of amoebocytes. From his description and figure of this "muff" the present author finds great similarity between it

and the sheaths noted surrounding dead and dying larvae of P. filicollis in the copepods C. albidus and E. serrulatus speratus.

It was noted earlier that procercoids of P. filicollis occasionally escape ensheathment and grow and develop normally. No procercoid greater than 44 µm. long was ever noted to be ensheathed, and it may be possible that larvae, if they manage to attain this size unmolested by host cells, will remain unmolested and develop normally. Thus Hynes & Nicholas (1958) have shown that acanthocephalan larvae in normally unsuitable amphipod hosts will not be attacked by host blood cells once they attain a certain stage of development. It has been suggested by Salt (1963) that the activity of cercaria in insect hosts might be responsible for their ability to resist attack by host cells. Thus it is possible, but unlikely, that the P. filicollis procercoids which escape ensheathment are also the most active.

Thus, considering the above studies of inhibited procercoid development in unsuitable copepod hosts, two levels at which host specificity may act within the copepod haemocoel can be noted. Firstly, as shown by the inhibited development of the powan proteocephalid procercoid in C. albidus and E. serrulatus s.s., the composition of the haemolymph is in some way unfavorable to invading procercoids.

Secondly, as exemplified by the inhibition of P. filicollis in C. albidus and E. serrulatus speratus, the copepod is intolerant of the parasite's presence and apparently physically rejects the parasite. The fact that procercoids of P. filicollis, which escape the defence reaction of E. serrulatus speratus, develop normally, would indicate that the host's intolerance of the parasite does not necessarily mean that the host's haemolymph is nutritionally at least unfavorable for normal procercoid development.

For a procercoid to develop in the haemocoel of a copepod host it must find the haemolymph favorable and must fail to stimulate a defence reaction by the host. In the case of Cyclops albidus, when infected with the powan procercoid no reaction ensued although parasite death occurred, while a reaction towards invasion with P. filicollis was elicited. Obviously the latter parasite was recognised by the host as foreign and was subsequently attacked by host blood cells.

There remains, however, much work to be done on such host/parasite systems. The electron microscope could examine in detail the morphological changes in the ultrastructure of unhealthy procercoids in unsuitable hosts and also examine the host/parasite interface within the sheaths described above. Any evidence of digestion of enclosed parasites could probably be discovered using this technique, although Salt (1963) has suggested that encapsulated insect

parasites probably die from lack of oxygen. Use of modern micromanipulative techniques could also be employed to investigate the reactions of copepods to developing procercoids injected directly into the copepod haemocoel. By use of the above technique much more information about host/parasite relationships between helminth parasites and their invertebrate intermediate hosts could be gained.

SUMMARY

(1) The copepods Eucyclops serrulatus s.s. and Cyclops albidus were shown to be unsuitable for procercoid development of a species of Proteocephalus found in the powan, Coregonus lavaretus of Loch Lomond. The young procercoids died in the haemocoel without eliciting any obvious host response.

(2) The copepods Cyclops albidus and Eucyclops serrulatus speratus were shown to be unsuitable for procercoid development of P. filicollis a parasite of the three-spined stickleback, Gasterosteus aculeatus. Procercoid death in these hosts was accompanied by or assisted by the formation of a sheath, of what was probably, host blood cells around the larva.

(3) Factors related to onset and causes of procercoid death in unsuitable copepod hosts are discussed in relation to the problems of host specificity in procercoid/copepod systems.

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SECTION 7

Egg production of Schistocephalus solidus (Müller) with observations on egg viability and procercoid development.

(with 6 figures in the text)

INDEX

INTRODUCTION

Orr & Hopkins (1969) established the entire life cycle of Schistocephalus solidus in the laboratory. One of the few drawbacks of their technique, as they themselves admitted, was that faeces from birds, even on a low nitrogen diet, contain a lot of uric acid crystals, the majority of which cannot be separated by sieving as the crystals and eggs have a similar size range. The authors suggested that 'removal of the mature worm from the bird and subsequent culture in a balanced saline solution for a few hours, although giving fewer eggs per worm, will prove a better method'. Indeed Parsons (1968) has recorded substantial egg returns from S. solidus during a 6 hour incubation period in Hanks' after maturation in avian and mammalian hosts for 2, 3 and 5 days. An investigation of the egg output of S. solidus recovered after 2 days from chickens over the same period in Hanks' constitutes the first part of the present study. In the second section, the viability, development, and infectivity of the eggs produced in vitro and the development of S. solidus procercoids in the copepods Eucyclops serrulatus s.s. (Fischer) and Eucyclops serrulatus speratus (Lilljeborg) is described. In the final section the ability of S. solidus eggs to develop in various concentrations of sea water is described,

together with a preliminary study of the importance of protonephridia in the salt and water balance of the eggs and larvae.

MATERIALS AND METHODS

1. Egg Production

(a) Infection of chickens

Plerocercoids of Schistocephalus solidus, removed from the body cavity of Gasterosteus aculeatus caught locally, were, after weighing in aluminium foil pouches, force-fed to 2-4 weeks old chickens. Food, a commercially supplied 'chicken mash diet', and water, was available to the chicks at all times, but was withheld from 18 h before infection until 2 h after infection. Since eggs were not to be collected from the faeces the feeding of the chicks on a low protein diet was unnecessary.

(b) Worm recovery

The chickens were killed 48 h after infection by breaking the neck, and the gut from gizzard to anus was removed. The now mature worms were recovered and weighed before incubation.

(c) The incubation medium

The medium (approx. pH7) was prepared 2 days prior to incubating the worms using 95 ml of Hanks' saline buffered with 5 ml of a 1.4% $NaHCO_3$ solution in a 200 ml

stoppered bottle gassed for 30 min. with a 95% air/5% CO_2 mixture. Two hours prior to incubation 20 ml of medium was transferred to a 3" by 1" glass tube, firmly stoppered and placed in a shaking water bath (oscillating over 10 cm at approximately 120 oscillations per min., for 15 seconds at 15 second intervals) at 40.5°C (the rectal temperature of a chicken).

(d) In vitro incubation

After recovery, the mature worms were placed in twos or threes, or as in later runs, singly in the tubes, which were again gassed and returned to the water bath for a 6 h period.

(e) Egg recovery

After 6 h each tube was opened, the pH of the medium measured in some runs, and the worms, with the aid of forceps, shaken to dislodge adherant eggs and removed. The tube was allowed to stand for 5 min. to allow the eggs to settle. The supernatant saline was drawn off with a pasteur pipette without disturbing the eggs and replaced with tap water at room temperature.

(f) Egg counting

The contents of each tube were transferred separately with washing to a 100 ml beaker. After rapid transfer of the eggs and water from one beaker to the other 7 to 8 times, the eggs in 3 5 ml samples were counted, and the total number of eggs produced in each tube calculated.

2. Egg viability and development

Batches of 300 to 600 eggs in either tap water or various dilutions of sea water were in a number of experiments set to develop in the dark in 65 ml jars in an orbital incubator at 23°C and subjected to 53 oscillations per min. for 5 min. every $^{1}/_{2}$ h. A 37μ sieve (Endecotts, London) was used to manipulate the eggs. The supernatant in each jar was periodically removed and replaced with the appropriate solution. During the experiments some eggs were removed with a pasteur pipette from the jars and transferred to a McMaster slide for microscopic examination to determine the amount of development. Four stages of development were used; stage A, unembryonated, stages B and C, partially developed, and stage D fully embryonated (Fig. I).

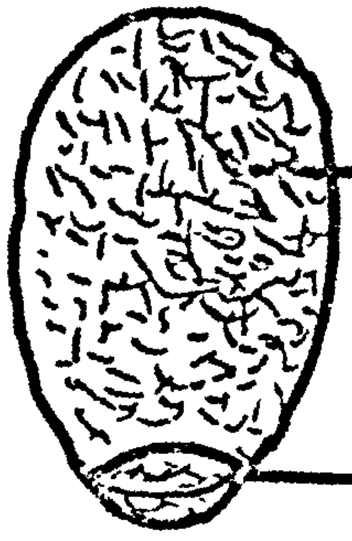
3. Egg hatching

Fully developed eggs were exposed to either daylight

Figure 1

The successive stages in the development of the egg of Schistocephalus solidus (from Mason 1965). * egg with embryos possessing distinct hooks, have, although not fully developed, been classed as stage D, rather than stage C.

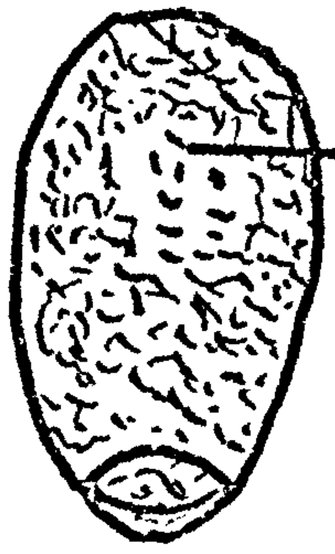
STAGE A



YOLK MATERIAL

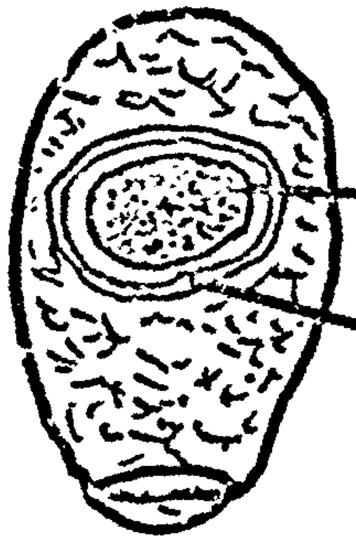
OPERCULUM

STAGE B



CLEAR AREA

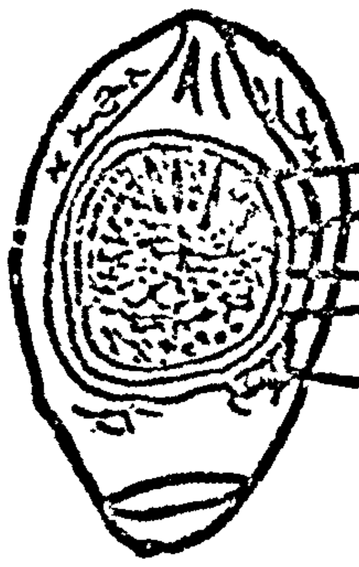
STAGE C



EMBRYO

LARVAL COATS

STAGE D



HOOKS

LARVAL COATS

YOLK DEPOSITS

or artificial light to induce hatching.

4. Infection of copepods

Eucyclops serrulatus s.s., collected from a canal in Glasgow, and E. serrulatus speratus from the same canal at Clydebank were added to the vessels containing hatched coracidia. The copepods were maintained at 19°C and mounted periodically for microscopic examination. By moving the coverglass the copepod could be held in any desired position so that the procercoids present could be counted and measured with a camera lucida. From measurements of length and breadth the volume of procercoids was calculated assuming them to be cylinders. Copepods were identified using the key of Harding & Smith (1960) and kindly verified by Mr. Harris of the British Museum.

5. Flame cell activity of eggs and coracidia

Fully embryonated eggs (stage D) were mounted under oil for examination (X400). Similarly coracidia, hatched in water, were mounted in a 20% Ficoll (Pharmacia, Sweden) solution and examined. Ficoll is extremely viscous, yet osmotically inactive, and so slows down physically the movement of the coracidia. Using a hand tally and a

stopwatch the rate of beat of the cilia of the first flame cell observed in each egg and coracidium was determined.

RESULTS

(1) Production of Schistocephalus solidus eggs in vitro.

Worms incubated in two/s or three/s for the 6 h period produced large numbers of eggs (Table I). The mean egg output per worm was 24,496, and the average fresh weight of the plerocercoids administered to the chickens was 188 mg. Negligible amounts of organic debris were carried over from the bird gut resulting in extremely clean eggs. In two similar runs, with more than one worm per incubation tube, the final pH of the medium ranged from 6.6 - 6.8 (6 tubes measured).

Worms incubated singly also produced large numbers of eggs (Table 2). Although the numbers of eggs produced by different worms varied considerably, the mean egg output per worm was 26,790 while the plerocercoids administered to the chickens weighed on average 186 mg fresh weight. Eggs were again noted to be perfectly clean, and in three similar runs, with only 1 worm per tube, the final pH of the medium ranged from 6.4 - 7.0 (16 tubes measured).

Considering the 6 runs of the technique together, the 35 mature worms recovered from the chickens represented 84% of the plerocercoids initially administered. Worms were generally found 40% to 60% down the length of the bird intestine.

Table I The egg production of Schistocephalus solidus incubated in groups in Hanks's saline for 6 hours after 48 hours in vivo

	CHICKEN	FRESH WEIGHT OF PLEROCECOIDS INDIVIDUALLY MG.	TOTAL MG.	NUMBER OF EGGS PRODUCED $\times 10^3$
RUN I	1	210 140	350	64·1
	2	250 170	420	41·5
	3	190 150	340	50·5
RUN II	1	210 340	550	46·1
	2	350 150	500	65·3
	3	250 220	470	66·1
	4	160 150	310	39·7
	5	130 100 30	260	42·7
		MEAN 188		MEAN 24·4

Table II The egg production of Schistocephalus solidus incubated individually in Hanks's saline for six hours after forty eight in vivo

	CHICKEN	FRESH WEIGHT OF PLERCERCOID MG.	NUMBERS OF EGGS PRODUCED x10³
RUN I	1	240	5·6
	2	230	3·0
RUN II	1	170	27·1
		120	16·4
	2	130	15·8
	3	270	30·6
		180	31·2
	4	160	20·8
		130	19·7
RUN III	1	280	69·6
		220	38·4
	2	140	32·6
		100	11·6
	3	270	12·1
RUN IV	1	240	29·8
	2	230	47·5
	3	220	21·5
	4	220	35·6
	5	200	25·1
	6	170	15·9
	7	140	40·9
	8	120	8·5
	9	120	27·8
		MEAN 186	MEAN 26·7

(2) <u>The viability and relative embryonation rate of eggs from different worms</u>.

The development of the eggs produced <u>in vitro</u> by 9 worms matured singly in chickens (TableII, run 4) is indicated in Fig. 2. The percentage of eggs still at stage A on the 7th day, i.e. the percentage of inviable eggs, varied considerably. While many eggs produced by worm 3 failed to develop, practically every egg from worms 1 and 4 embryonated normally. The percentage viability was generally greater than 80%. The rate of development of eggs from different worms was also noted to vary considerably, eggs from worms 4, 7 and 8 being considerably slower to reach stage D than those of the other worms. The same differences in egg developmental rates were also apparent on Day 5. No connection between egg viability and rate of development was observed. Neither of the above variables could be correlated with the initial weight of plerocercoid administered.

(3) <u>The infection of the copepods</u> Eucyclops serrulatus s.s. <u>and</u> E. serrulatus speratus <u>with</u> Schistocephalus solidus <u>procercoids</u>.

It is clear from Fig. 3 that eggs produced <u>in vitro</u> from worms matured <u>in vivo</u>, after embryonation, released

Fig. 2 The development of the eggs produced by Schistocephalus solidus incubated individually in Hank's saline for six hours after forty eight hours in vivo. A B C and D represent the progressive stages of egg development (Fig 1).

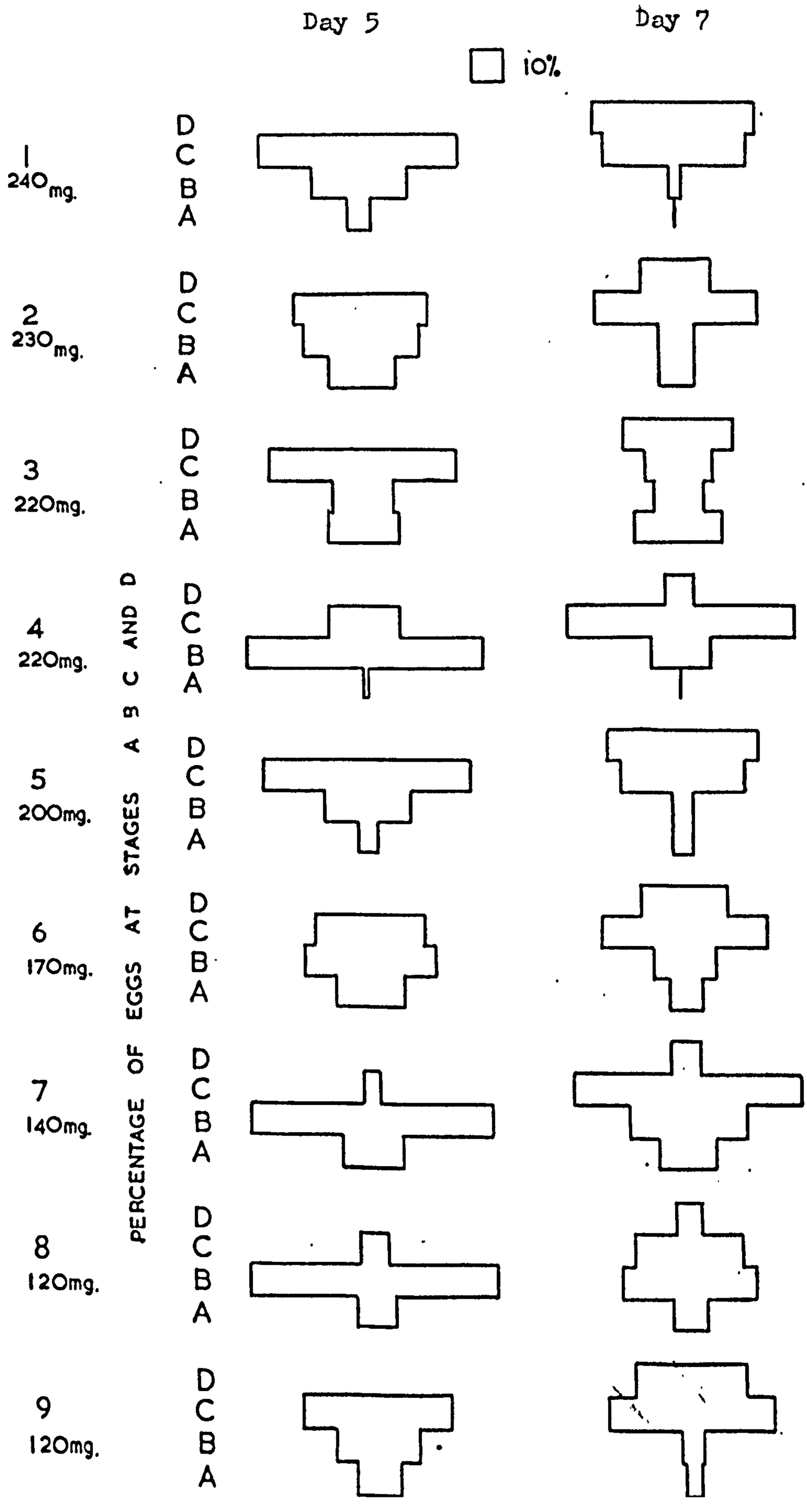
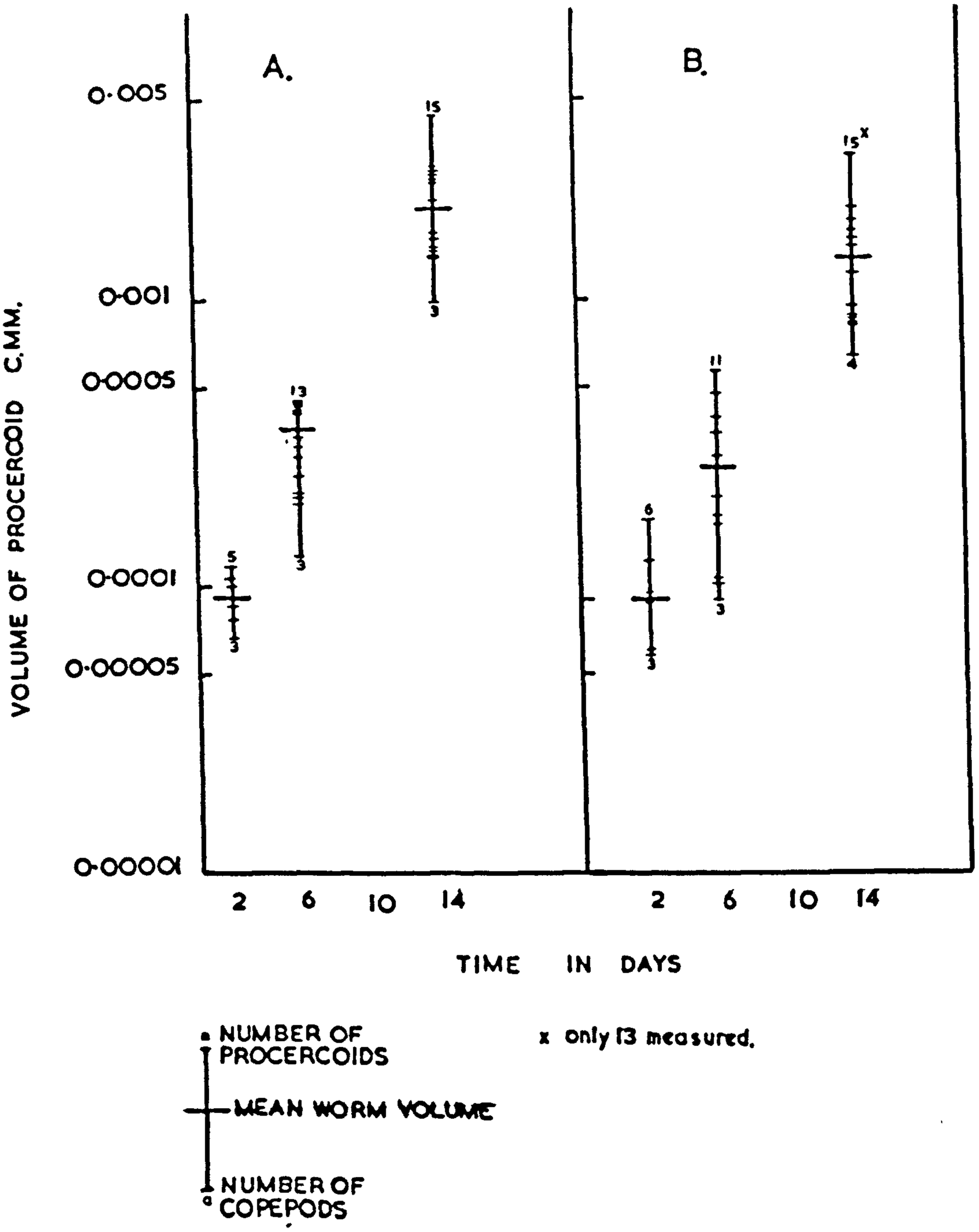


Fig.3 The growth of Schistocephalus solidus procercoids in Eucyclops serrulatus ss.(A)and Eucyclops serrulatus speratus (B)

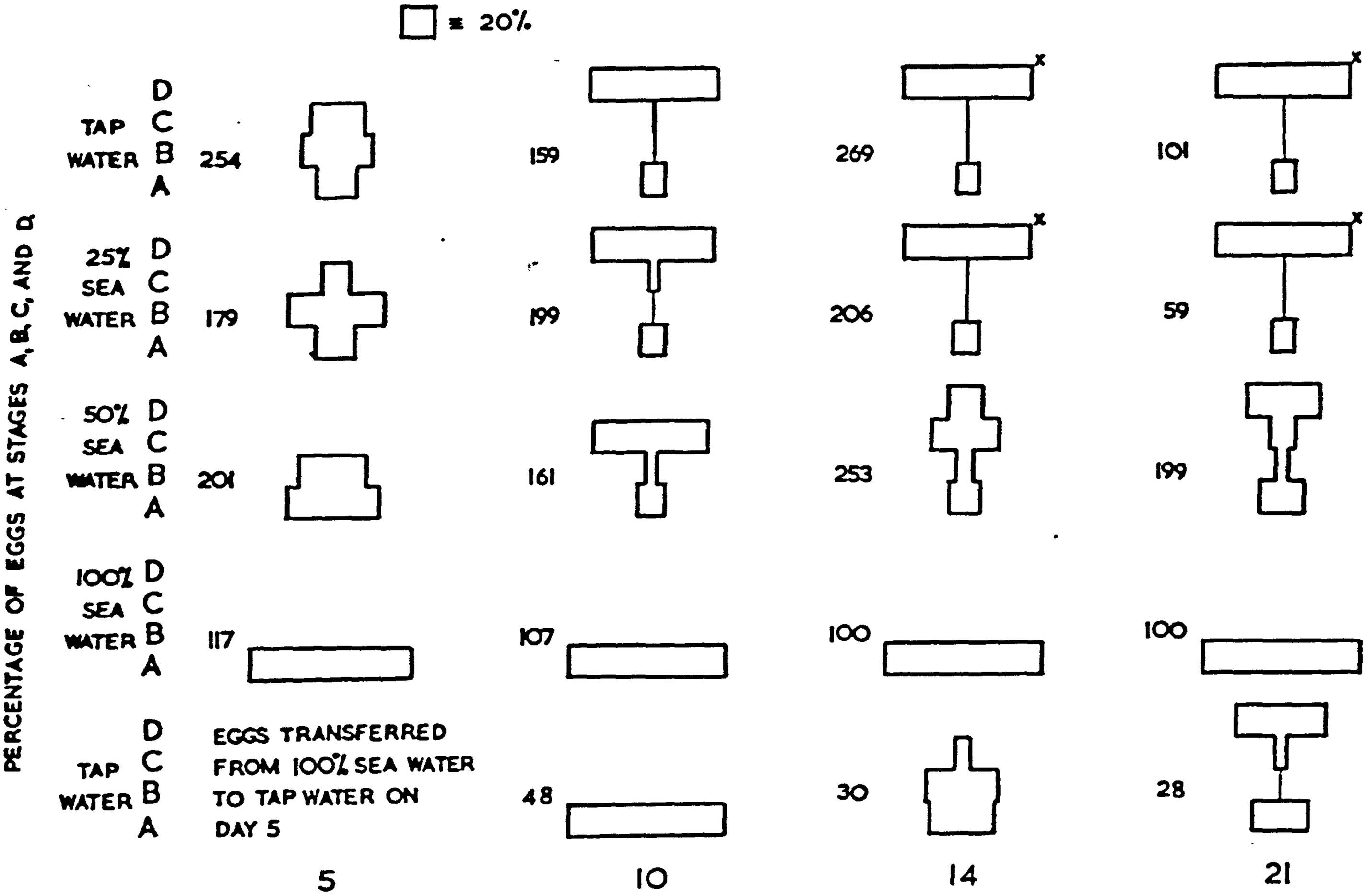


coracidia infective to copepods. Infection of both species, with 1 to 7 worms per copepod, was 100%. Procercoids grew at a similar rate in both species, although the greater variation in worm volume noted in E. serrulatus speratus, resulted in the smaller mean procercoid volume in this species compared to those developing in E. serrulatus s.s. on the 6th and 14th days. A procercoid 0.0027 C.mm. in volume on the 14th day measured 362 µm by 93 µm. All procercoids, in both species, had, by the 14th day, shed their cercomers, and were fully developed with calcareous corpuscles and the anterior indentations. All control copepods (100 of each spp.) proved negative for infection.

(4) The development of eggs in 25%, 50% and 100% sea water, and in tap water.

Over 80% of the eggs in 25% sea water and tap water were fully developed by 14 days (Fig. 4). Thereafter many of these eggs, classified as stage D, had in fact hatched. Eggs in 50% sea water developed more slowly; only 46% had reached stage D on the 21st day. All eggs in 100% sea water remained at stage A, there being no indication of development whatsoever. Occasionally the shell of such eggs collapsed, appearing concave instead of

Fig4 The effect of salinity on the development of Schistocephalus solidus eggs maintained at 23°C. A B C and D represent the four progressive stages of development indicated in Fig 1. The number of eggs examined is quoted to the left of each column.



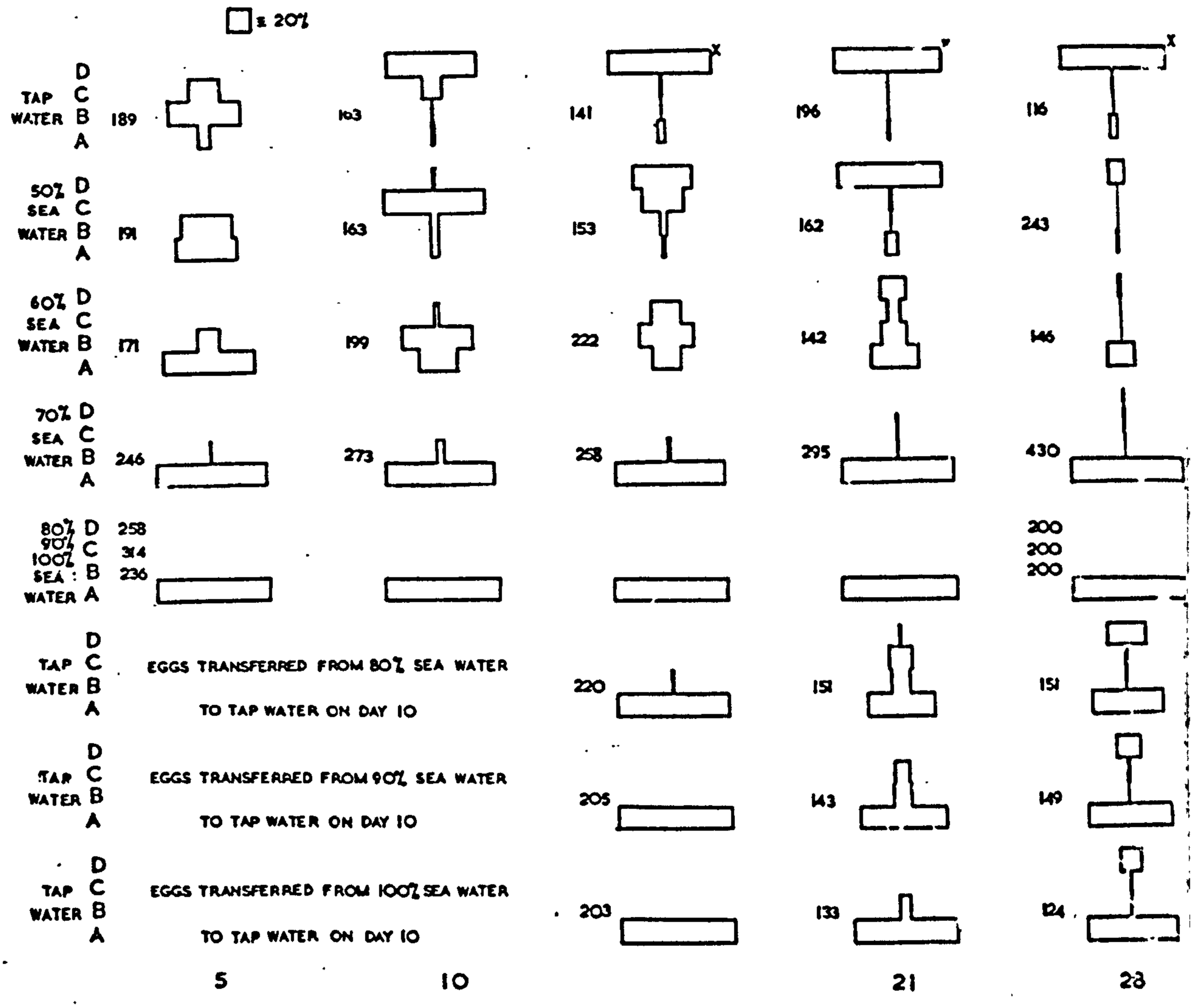
x Many of these eggs had hatched

convex on one side. Eggs transferred to tap water, after 5 days in 100% sea water, were still at stage A on day 10 after 5 days in tap water. By day 14, however, development was apparent, some eggs having reached stage C, while by the 21st day many eggs were fully developed.

(5) <u>The development of eggs in 50%, 60%, 70%, 80%, 90% and 100% sea water and in tap water.</u>

Eggs in tap water were mainly at stage D by 10 days and many eggs classified as stage D thereafter had in fact hatched (Fig. 5). Although development was much slower, over 90% of the eggs in 50% sea water were fully developed on day 21, but as in the previous experiment none hatched, with the result that over 80% of the eggs appeared abnormal on the 28th day. The oncospheres of such eggs were markedly shrunken and disorganised. As in tap water very few eggs remained undeveloped. Eggs in 60% sea water were even slower to develop; only 20% of the eggs were fully developed on the 21st day. The great majority of eggs in 60% sea water were abnormal on the 28th day. Many of the eggs in 60% sea water showed no development remaining at stage A. Very few eggs in 70% sea water developed at all, only one fully developed egg being noted. All eggs in 80%, 90%, and 100% sea water remained at stage A. Shell collapse

Fig 5 The effect of salinity on the development of Schistocephalus solidus eggs maintained at 23°C. A B C and D represent the four progressive stages of development shown in fig 1. The number of eggs examined is quoted to the left of each column.



X Many of these eggs had hatched.

of some eggs in 100% sea water was again observed occasionally. Eggs transferred to tap water after 10 days in 80%, 90% and 100% sea water developed, 30%, 23% and 19% respectively reaching stage D by the 28th day after 18 days in tap water, eggs transferred from 100% sea water being slowest to develop. The majority of all three of these groups of eggs, however, failed to develop in tap water.

(6) The infection of the copepod Eucyclops serrulatus using coracidia developed and hatched under various conditions.

Fully developed eggs from tap water, 12.5% sea water, and 25% sea water were exposed, after 14 days, to artificial light either in the solution in which they had developed or after transferance to a different solution (Table III). After 2 days copepods were added to each jar in which many coracidia had already hatched. The copepods placed with the coracidia hatched in brackish conditions, had been allowed to acclimatise to such conditions for 2 days.

Since copepods, exposed to coracidia which had developed and hatched in 12.5% and 25% sea water, became infected like the control copepods, exposed to normal coracidia, developed and hatched in tap water, it is clear that at least some of the former coracidia were infective. All

Table III The infection of Eucyclops serrulatus with procercoids of Schistocephalus solidus using coracidia developed and hatched under various conditions of salinity

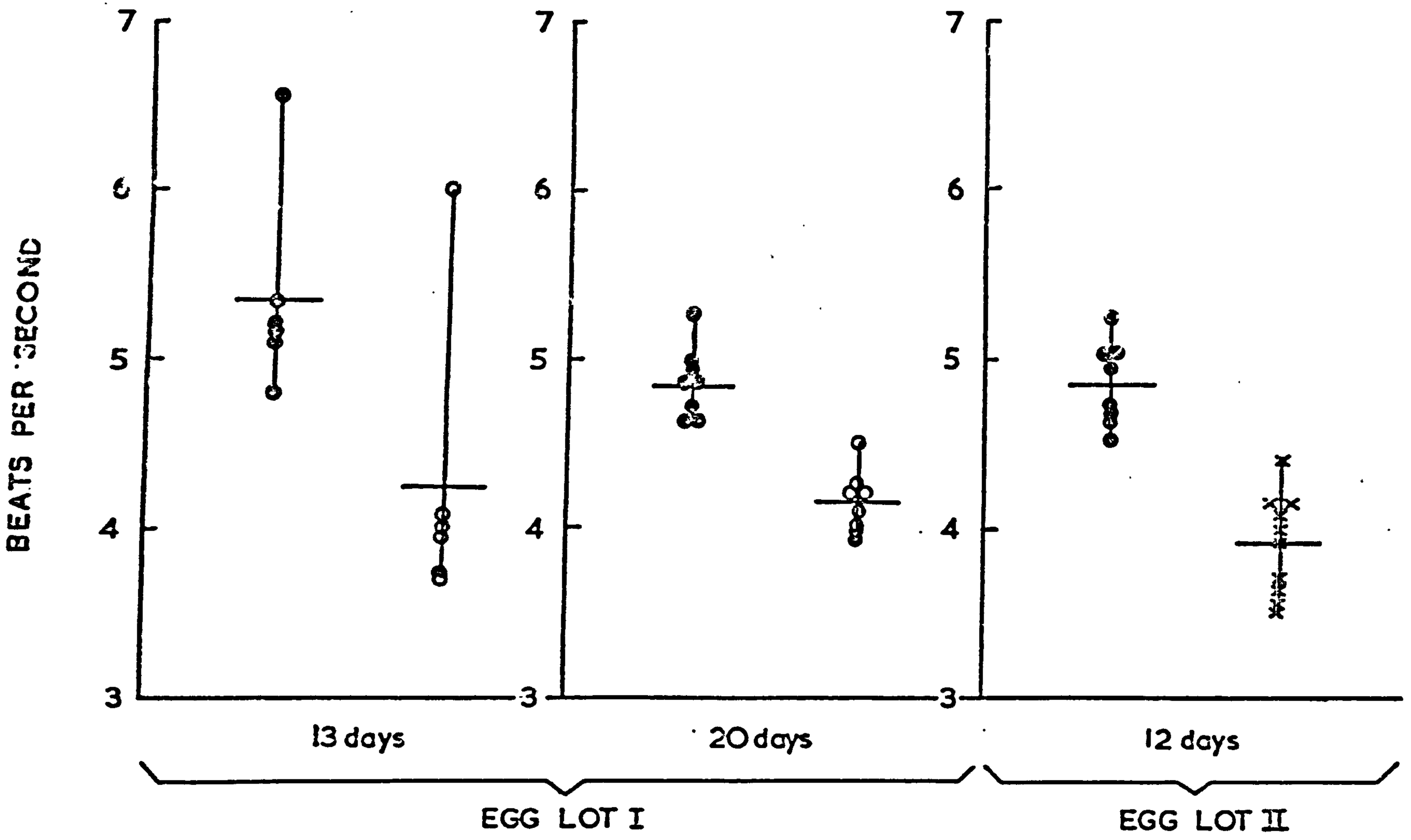
CONDITION OF EGG DEVELOPMENT	HATCHING	NUMBER OF COPEPODS EXAMINED	NUMBER OF COPEPODS INFECTED	NUMBER OF PROCECOIDS
TAP WATER	TAP WATER	3	3	10
	25% SEA WATER	2	2	2
12·5% SEA WATER	TAP WATER	3	3	12
	12·5% SEA WATER	3	3	4
25% SEA WATER	TAP WATER	3	3	7
	25% SEA WATER	2	2	10

larvae within copepods appeared normal.

(7) <u>The effect of increased external salinity on the flame cell activity of fully developed eggs.</u>

The mean beat rate of the cilia of the flame cells of eggs 3 hours after transfer to the two 25% sea water solutions and the 0.74% NaCl solution (Δ = 0.46 osmotically equivalent to 25% sea water), as shown in Fig. 6, were 4.2, 4.1, and 3.9 beats per second respectively while the equivalent rates in tap water were 5.3, 4.8, and 4.8 beats per sec. The overlap in the range of beat rates of the two groups of 13 day old eggs is considered to be misleading since the two results responsible are obviously abnormal. The rate of beat of the cilia of the flame cells of the 2 hatched coracidia in water was approximately 10-12 beats per sec.

Fig 6 The rate of beat of the cilia in the protonephridia of Schistocephalus solidus eggs in fresh water 25% sea water and 0·74% NaCl, the mean being shown in each case.



⊖, ○, and × represent the individual beat rates of eggs in fresh water, 25% sea water, and 0·74% NaCl respectively

DISCUSSION

By simply maintaining mature Schistocephalus solidus in buffered Hanks' at 40.5°C for 6 hours large numbers of eggs can be reliably produced. Plerocercoids (mean fresh weight 188 mg) after 48 hours maturation in a chicken produced in vitro a mean of 25,600 eggs (Tables I & II). Parsons (1968) using virtually the same in vitro technique, but maturing her plerocercoids in ducks found the average egg production per worm to be 25,000. She also noted that most eggs were produced during the first hour of incubation. Her eggs were 60% - 70% viable whereas the mean viability of eggs in fresh water in this study (Figs. 2, 4 & 5) was 89%.

Considering the high level of egg viability it is clear that most of the eggs produced in vitro were fertilised. It is unlikely that insemination of the receptacular could occur in the incubation tubes utilised as Smyth (1954) recorded that insemination in vitro occurred only when S. solidus were compressed within dialysis tubing but not when free in the culture medium. The infection of copepods (Fig. 3 & Table 3) and the normal development of procercoids indicates the normality of the eggs produced during the 6 hour in vitro incubation period.

Eucyclops serrulatus s.s., unlike the closely related

E. serrulatus speratus, has been recorded as a suitable intermediate host for S. solidus procercoids. (Nybelin 1919, Clarke 1954, Mason 1965). Although growth of S. solidus procercoids in E. serrulatus speratus was more variable than in the above species (Fig. 3), it seems justified to add E. serrulatus speratus to the list of 10 copepods already recorded as suitable hosts for S. solidus procercoids (Orr & Hopkins, 1969).

The development and hatching of infective coracidia in 25% sea water (Table 3) verifies the unqualified statement of Hilliard (1960) that Schistocephalus solidus is euryhaline, its eggs being able to develop under brackish conditions. Thus S. solidus may well cycle in brackish water and so explain the relatively high incidence of plerocercoids reported by Markowski (1966) in three-spined sticklebacks living in water where the salinity ranged from 1.3% to 14.3% (i.e. from 3.7% - 41.3% sea water). Although Eucyclops serrulatus and Acanthocyclops viridis, both suitable hosts for S. solidus procercoids, have been reported from brackish water (Saltynska 1964) it seems possible that S. solidus, being catholic in its choice of copepod hosts, might well utilise more typical brackish or marine species under these conditions.

It is clear, however, that S. solidus eggs cannot develop normally and hatch in marine or semi-marine conditions (Figs. 4 & 5). S. solidus eggs developed normally in hypotonic fluids (Hilliard (1960) estimated the tonicity of Diphyllobothrium dalliae coracidia to be = 1% NaCl which is ≡ 33% sea water) but not in hypertonic solutions. Many unembryonated S. solidus eggs, however, are tolerant of hyperosmotic solutions as indicated by their ability to develop in fresh water after exposure for 5 days (Fig. 4) and 10 days (Fig. 5) to sea water.

The activity of the flame cells of eggs transferred to 25% sea water or 0.74% NaCl was lower than that of the eggs remaining in fresh water (Fig. 6). The egg shell of S. solidus is probably freely permeable to water and ions like that of Fasciola hepatica (Rowan 1962, Wilson 1967, 1969). However, also like F. hepatica (Wilson 1967), the lippo-protein vitelline membrane lying immediately within the shell and surrounding the unhatched coracidium is unlikely to be freely permeable to water and salts. As the eggs were transferred to hyposmotic solutions (Hilliard 1960) water would not be withdrawn from the coracidium. Thus although the reduction of the flame cell activity (Fig. 6) would suggest an osmoregulatory rôle for the

protonephridia of the S. solidus eggs, theoretical evidence for this rôle is lacking. Indeed Wilson (1967) has shown that the flame cell activity of hatched miracidia of F. hepatica is unaffected by changes in the external osmotic pressure. Considering the differing opinions of other workers (Beadle 1934, Pantin 1947, and Carter 1961) one is forced to agree with Schwabe and Kilejian (1968) who conclude that evidence for an osmoregulatory role for protonephridia is 'comparatively meagre'. The increased flame cell activity of hatched coracidia probably reflects either an increased intake of water by the coracidium now unprotected by the vitelline membrane or simply the increased metabolic rate of the now free-swimming coracidium. Wilson (1969) noted that the flame cell activity of miracidia of F. hepatica increased just prior to hatching.

In vitro and in vivo egg production

The mean egg yield of plerocercoids (mean fresh weight 188 mg) after 48 h in a chicken , when placed in culture for 6 hours was 25,600. This high egg yield from a simple procedure which avoids the laborious and inefficient process of sieving faeces from infected birds should suffice for laboratory maintenance of S. solidus and provide

enough eggs for studying the physiology and development of pseudophyllidean eggs.

SUMMARY

(1) A technique for the *in vitro* production of viable *Schistocephalus solidus* eggs is described. Plerocercoids (mean fresh weight 188 mg) after 48 h maturation in a chicken produced *in vitro* in 6 h 25,600 eggs. The mean viability of the eggs was 89%.

(2) The percentage viability and rate of development of eggs from different worms is compared.

(3) The copepod *Eucyclops serrulatus speratus* was shown to be yet another suitable host for procercoid development of *S. solidus*.

(4) *S. solidus* eggs developed normally in 25%, abnormally in 50%, 60% and 70%, and not at all in 80%, 90% and 100% sea water. Eggs could recover and develop in fresh water after 5 days in sea water. The ecological and physiological significance is discussed.

(5) The activity of the flame cells of unhatched coracidia was reduced in 25% sea water and 0.74% NaCl, and increased after hatching.

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